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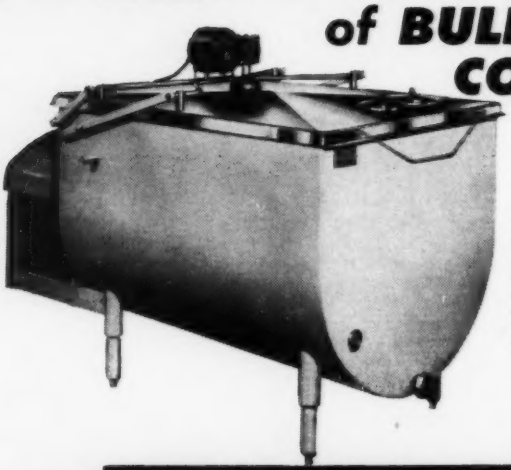

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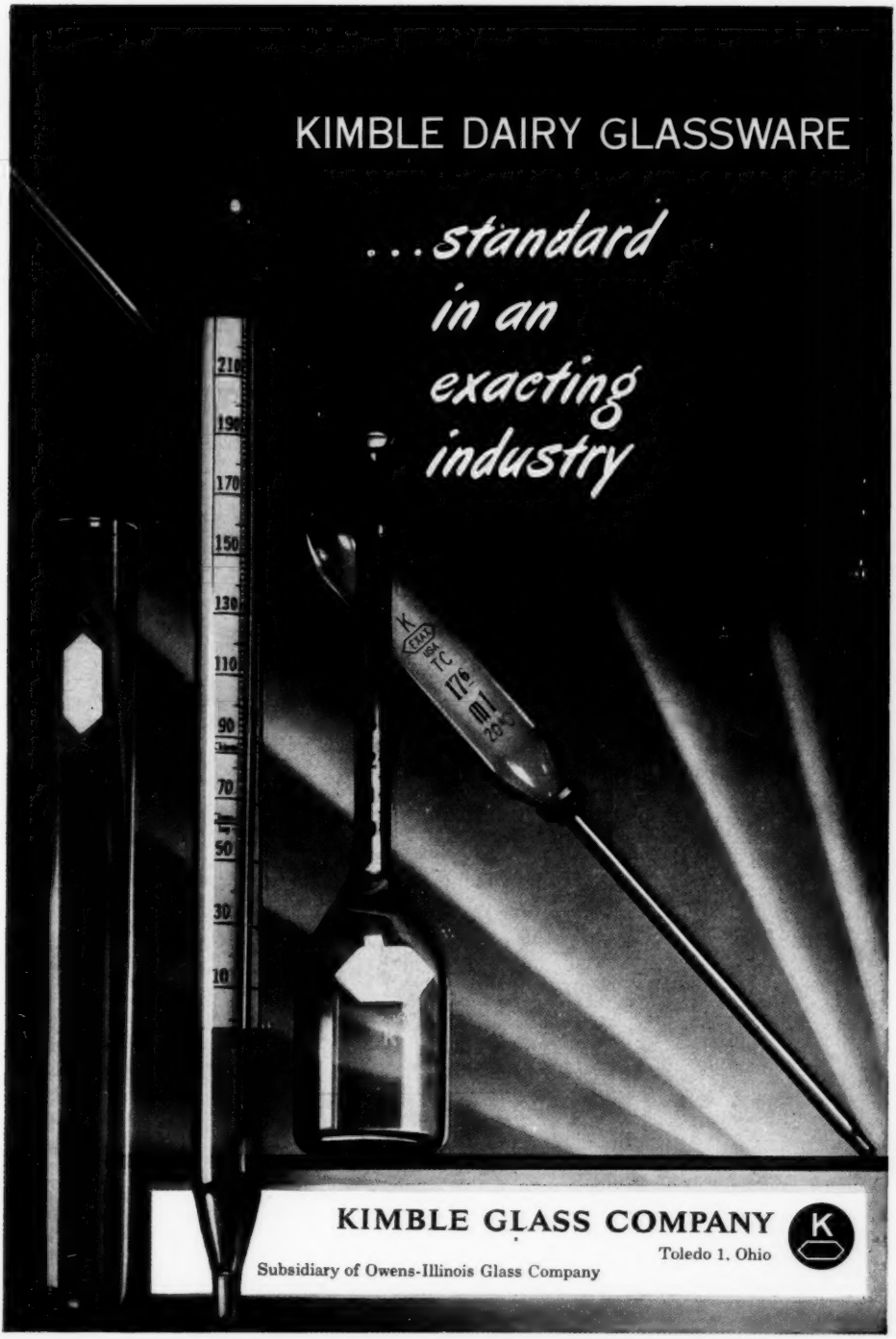

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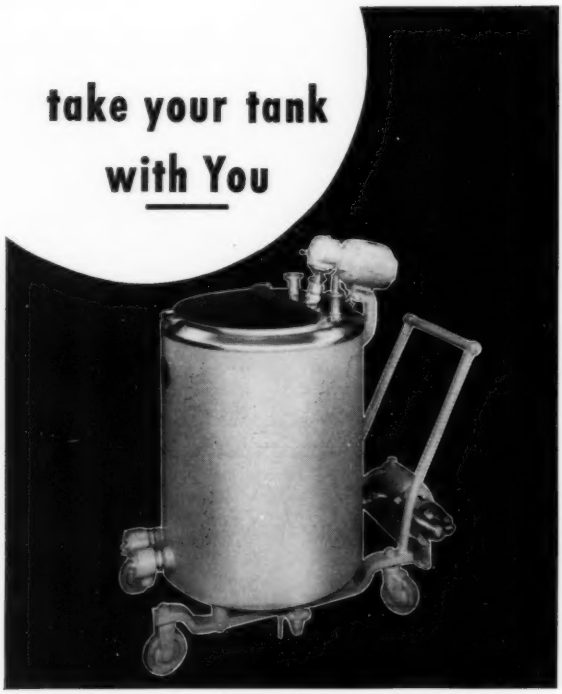

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVI APRIL, 1953 NUMBER 4

SLACKED LIME AS A PREVENTIVE OF SCOURS FOR WHEY-FED CALVES[1]

DONALD C. BROWN, JAMES READ, AND H. S. WILLARD
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There is need for greater utilization of whey. For many cheese plants whey disposal is a major problem. Some plants return whey to the farmers for feeding calves and pigs. Some of the larger plants are equipped to dry whey and to manufacture products such as whey protein, whey cheese, lactose, lactic acid, riboflavin, alcohol, and vinegar.

Dried whey contains certain nutritional food elements, as shown by the following comparison with nonfat dry milk solids.

	Dried Whey (%)	Dry Milk Solids (%)
Ash	8.6	7.8
Organic Solids	91.4	92.2
Fat	1.1	1.2
Protein	13.0	34.7
Lactose	73.9	50.3

There are reports in literature of the laxative quality of whey. Wallace *et al.* (10) stated that incidence of scours increased with the level of dried whey in feed mixes. Daniel and Harvey (3) showed that increase in scouring is due to the soluble salts of whey. Fisher and Sutton (5) reported on the laxative nature of lactose.

There are several preventive measures against nutritional scours. Recent reports show that addition of Aurofac or crystalline aureomycin decreased incidence of scours (1, 2, 4, 7). Slacked lime probably is the cheapest substance suggested as a cure for scours. According to Winslow (12) "Solutions of lime are a direct sedative to the stomach, and mixed with milk equal volumes prevent the rapid coagulation of casein, lessen the formation of large, tough curds in the stomach, and assist the retention and digestion of milk. Syrup of lime is twenty-four times stronger in calcium hydrate than lime water, and is more astringent. It may be given to foals and calves suffering from indiges-

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[1] Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 26.

tion and diarrhea.'' Hutyra *et al.* (6) also suggested use of lime water as an astringent and neutralizer for calves.

This paper reports a series of studies designed to investigate the value of slacked lime as a check to nutritional scours in whey-fed calves. Nutritional scours was distinguished from infectious scours by observation and included the calves which scoured but still maintained a normal appetite and body temperature.

EXPERIMENTAL PROCEDURE

For the most part, male and female calves of the Holstein breed were used in these experiments. Twenty-six animals were housed in individual pens and bedded with straw or shavings. In addition to the whey as a substitute for whole milk, all calves received native hay and dry concentrate mixture made up of the following ingredients:

300 lb. rolled oats
300 lb. rolled barley
100 lb. soybean oil meal (41 per cent)
100 lb. bran
100 lb. dehydrated alfalfa meal or pellets
9 lb. salt
9 lb. bone meal

The mixture contained approximately 16 per cent crude protein.

The calves were not on experiment at the same time but were placed on test as space became available. Seven groups were provided:

Group 1: Whole milk discontinued at 10 days of age. Whey introduced on fourth day of age. Calves on all-whey diet at 10 days of age.

Group 2: Whole milk discontinued at 3 weeks of age. Whey introduced on fifteenth day of age. Calves on all-whey diet at 20 days of age.

Group 3: Whole milk discontinued at 2 weeks of age and slacked lime added at start of scouring. Whey introduced on eighth day of age. Calves on all-whey diet at 15 days of age.

Group 4: Whole milk discontinued at 3 weeks of age and slacked lime added at start of scouring. Whey introduced on fifteenth day of age. Calves on all-whey diet at 21 days of age.

Group 5: Whole milk to 45 days of age, no whey, without lime.

Group 6: Whole milk to 60 days of age, no whey, with lime added at start of scouring.

Group 7: Whole milk to 30 days of age. Whey introduced on fifteenth day of age. Calves on all-whey diet at 30 days of age.

Group 1 and 2 received no lime. An attempt was made in group 2 to find out if faster gains might be attained on whey when the whole milk period was extended from 10 to 20 days of age. Group 1 was fed the liquid portion of the ration according to the following daily schedule (calf left on cow 3 days):

Age of Calf *(Days)*	Whey Powder *(lb.)*	Water *(lb.)*	Whole Milk *(lb.)*
4	0.2	1	6
5	0.4	3	5
6	0.6	5	4
7	0.8	7	3
8	1.0	7	2
9	1.0	8	1
10	1.0	9	0

11th to 40th day—fed same as 10th day—off whey 60th day.

The above schedule called for 39 lb. of whole milk and 52 lb. of whey powder to 60 days of age.

For group 2, the whole milk feeding continued until 20 days of age, 1 lb. of whey powder being introduced into the milk daily at 15 days of age. The whole milk was withdrawn between 20 and 23 days after birth and whey powder in water substituted in its place. Whey powder in water then was fed until the calf was 60 days of age. The schedule called for about 200 lb. of whole milk and 40 lb. of whey powder.

The milk or milk substitute feeding procedure for group 3 was the same as for group 1 except that the whole milk feeding was advanced 1 week and the calves were on all-whey liquid feeding at 20 days of age. The schedule called for 100 lb. of whole milk and 49 lb. of whey powder. When scouring took place, ¼ teaspoon of slacked lime was stirred into the whey mixture at each feeding until the scouring stopped.

The milk or milk substitute feeding procedure for group 4 was the same as for group 3 except that the whole milk feeding was advanced 1 week and the calves were on an all-whey liquid feeding at 21 days of age. The schedule called for 160 lb. of whole milk and 40 lb. of powdered whey. Slacked lime was added to the whey mixture whenever the calves scoured. Lime feeding was continued until the scouring stopped.

Groups 5 and 6 were fed whole milk to 60 days of age but in different amounts. As group 5 scoured, lime was added to the milk until the scouring stopped. Groups 5 and 6 were considered as control groups.

The amount of whole milk fed to group 7 was similar to that fed group 2, but the whey was introduced more slowly and less whey was fed. Whole milk was fed to 30 days of age with remade whey gradually introduced into the milk from the fourteenth day of age with the following daily schedule:

Age of calf (*Days*)	Whey powder (*lb.*)	Water (*lb.*)	Whole milk (*lb.*)	Total fluid milk products (*lb.*)
14	....	....	10	10
15	0.1	0.9	8	9
16	0.2	1.8	6	8
17	0.3	2.7	6	9
18	0.4	3.6	6	10
19 to 30	0.5	4.5	5	10
31	0.5	4.5	4	9
32	0.6	5.4	2	8
33 to 60	0.6	5.4	0	6
Total per calf	25.0	20.0	205	430

One calf scoured for three days beginning at 31 days of age. At this time a commercial enzyme furnished by George A. Jeffries Company was included with the remade whey until scouring stopped. Another calf scoured for 2 days beginning at 10 days of age. The same enzyme was used with the milk until scouring stopped.

RESULTS

TABLE 1

Milk and remade whey consumed, daily gains, and scouring to 60 days of age.

Lot No.	No. calves	Total whole milk	Total remade whey	Av. initial weight	Av. daily gains	Age at start of scouring	No. days scouring	Lime fed
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*days*)		
1	3	39	392	90	.45 ± .12	7	40	No
2	5	201	395	90	.65 ± .22	20	40	No
3	6	103	432	84	.80 ± .21	21	5	Yes
4	5	165	402	88	1.11 ± .14	30	3	Yes
5	4	359	.....	91	1.00 ± .35	1 calf for 1 day		No
6	3	475	.....	82	1.14 ± .27	13	4	Yes
7	4	205	250	95	.97 ± .29	[a]		

[a] One calf scoured 3 days at 31 days of age — commercial enzyme fed.
One calf scoured 2 days at 10 days of age — commercial enzyme fed.

Growth and scouring data with and without use of lime are summarized in Table 1. Lot 2 showed greater growth than lot 1, probably because more whole milk was fed. Both lots scoured continuously on whey. However, lot 3 grew faster than lot 2, even though less whole milk was fed. When more whole milk was fed, as in lot 4, the gains were improved over lot 3 and there were fewer days of scouring. Lots 5 and 6 are considered as controls because only whole milk was fed as the liquid portion of the ration. The two lots might be combined except for the fact that more milk was fed to lot 6 than to lot 5. More scouring occurred in lot 6, and lime was administered. Greater gains were made in lot 6, indicating that the scours were not severe enough to retard growth. It is impossible to show in lots 5 and 6 the influence of lime on scouring, be-

cause the group which scoured received the lime. To make the data comparable, there should have been a group which scoured and received no lime.

However, the data indicated that of the calves on whey, eight scoured continuously without lime, and eleven scoured from 3 to 5 days after lime was added to the milk when scouring began. Scouring in lot 7 was stopped apparently by a commercial enzyme. One calf scoured at 31 days of age when almost entirely on remade whey. One calf scoured at 10 days of age before the whey feeding was started.

DISCUSSION

Animals in lots 4, 5, and 6 may be considered sufficiently similar in growth to show the value of whey as a substitute for whole milk in the ration. Lots 5 and 6 were fed 405 lb. of whole milk and made an average daily gain of 1.05 lb. Lot 4 was fed 165 lb. of whole milk and 40.2 lb. of whey powder. By placing a value on the whole milk replaced, one may obtain a comparable value for whey powder fed this lot. If the whole milk (4% fat) is valued at $4.00 per hundredweight, then whey powder would have a value of 23.9 cents per pound ($\frac{240 \times \$0.04}{40.2} = \$0.239$). The data from group 7 would give whey a monetary value of 32 cents per pound ($\frac{200 \times \$0.04}{25} = \$0.32$).

Results indicate that remade whey should not be introduced into the ration until the calf is at least 2 weeks of age and that the change to an all-whey liquid diet should be gradual.

It was observed that in the groups to which no lime was fed, the calves stopped scouring in 2 to 4 days after whey was eliminated from the ration. It was observed also that when scouring was stopped by feeding lime, there was no recurrence of scouring. Remade whey was palatable and the calves fed whey seemed to eat grain sooner than the calves fed whole milk. Whey-fed calves were always a bit loose even when not scouring.

Calves fed 165 lb. of whole milk and 40.2 lb. of whey powder (Lot 4, Table 1) made normal growth. The average daily gain for 106 calves at the Wyoming Experiment Station (12) for the first 60 days from birth was 1.15 ± 0.22 lb. Data reported by Morrison (8) for 387 Holstein calves showed an average daily gain of 1 lb., whereas data reported by Ragsdale (9) for 237 Holstein calves showed an average daily gain of 0.97 lb. for the same period.

SUMMARY

Studies involving 30 Holstein calves were carried out to formulate the best system of starting and continuing calves on reconstituted whey to 60 days of age. Approximately normal rates of growth were obtained when whole milk was continued for 2 weeks, followed by a gradual change to remade whey, provided that when scours occurred they were stopped by the addition of slacked lime (¼ teaspoon) to each feeding of remade whey.

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A COMPARISON OF VARIOUS DIGESTION TRIAL TECHNIQUES WITH DAIRY CATTLE [1]

E. A. KANE, R. E. ELY, W. C. JACOBSON, AND L. A. MOORE
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Recently there has been an increased interest in several inert materials that may be used to determine the digestibility of the various constituents in a feed by the "ratio technique." The use of chromic oxide as an inert material in digestibility studies was first proposed by Edin (14). A discussion of the experimental methods used was published in English by Edin, Kihlen, and Nordfeldt (15).

Satisfactory results with the chromic oxide ratio technique have been reported also by Hamilton *et al.* (22); Andersen (1); Andersen and Frederiksen (2); Skulmowski *et al.* (40) using sheep; by Barnicoat (5) and Lloyd (30) with swine; by Olsson *et al.* (34) with horses; by Kane *et al.* (26, 27) with cows; by Schurch *et al.* (39) with rats; by Irwin and Crampton (24), Kreula (29), and Virtanen (43) with humans; and by Chanda *et al.* (7) with goats.

Barnicoat (5) obtained consistently low digestion coefficients with sheep and calves when the chromic oxide was given in a gelatin capsule. Crampton and Lloyd (10) reported that the chromic oxide may be accurately used in determining the digestibility of a sheep ration when it is premixed with ground grain but not when it is given separately, as was necessary on a ration composed solely of roughage.

Jarl (25) felt that there was a constant relation between the chromic oxide and the various nutrients in the ration and feces; hence a variation in feces quantity on two successive days would not affect the calculated digestibilities. Recent data of Kane *et al.* (28) showed a rhythmic variation in the concentration of chromic oxide and in lignin in individual passages of feces throughout a 24-hour period. Andersen and Winther (3) found that the ratio of chromic oxide to the various nutrients in the feces varied between that voided during the day and the night but was not always higher for one than the other, and these investigators suggested that the partial collection of feces samples be made at intervals throughout the entire 24-hour day.

Lignin has been reported to be indigestible by steers (11); by sheep (20); by cows on a mixed feed and by sheep on dried sudan grass (16); by sheep on clover-timothy hay (17); by sheep on mixed feeds (40); by sheep on Korean lespedeza hay cut at four stages of maturity (9); by steers on pasture forage (18, 19); by cows on a ration of alfalfa silage, corn silage, and grain; and by cows on a ration of alfalfa hay, corn silage and grain (27). However, various

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[1] From a portion of a thesis presented by R. E. Ely to the Graduate School of Michigan State College in partial fulfillment of the requirements for the Ph.D. degree.

degrees of digestibility of lignin have been reported also (6, 11, 12, 13, 21, 31, 35).

An indigestible plant pigment, or group of pigments, that is extracted with 85 per cent acetone has been reported by Reid *et al.* (36, 37). The recovery of these plant pigments in the feces was very close to 100 per cent with all of the materials studied. Other workers also have reported satisfactory results with the plant pigment ratio technique (8, 32, 33).

Cook and Harris (8) reported low plant pigment recoveries in the feces of sheep grazing big sagebrush and black sage. It is significant that the same workers secured close checks between the lignin ratio method and the plant pigment ratio method when using alfalfa hay.

No studies have been reported which have made comparisons of more than two inert materials in the same digestion trial when using the same animals receiving the same feed.

It was the object of this experiment to make a comparison of the digestion coefficients determined with milking dairy cows fed orchard grass hay cut at four stages of maturity. The following digestion trial techniques were used: (a) total fecal collection technique, (b) chromium ratio technique (total collection), (c) plant pigment ratio technique (total collection), (d) lignin ratio technique (total collection), (e) lignin (corrected for nitrogen) ratio technique (total collection), (f) chromium ratio technique (grab samples), and (g) plant pigment ratio technique (grab samples).

EXPERIMENTAL METHODS

The orchard grass hay used in this study was cut from a second growth of orchard grass that had been sprayed previously with 2,4-D for the control of weeds and clover. Two applications of 100 lb. and 150 lb. of ammonium sulfate per acre were made approximately 1 month and 1 week, respectively, before the first experimental cutting. Grass at different stages of maturity was harvested at approximately 2-week intervals as follows:

Stage 1, 6 to 7 in. high (pasture), cut May 19, 1949.
Stage 2, 8 to 10 in. high (late pasture), cut May 31, 1949.
Stage 3, 10 to 12 in. high (early hay), cut June 14, 1949.
Stage 4, 12 to 14 in. high (mature hay), cut June 27, 1949.

The first cutting was made with a forage field chopper and blown directly into wagons and hauled to the barn for artificial drying. The last three cuttings were left in the swath for approximately one day, windrowed with a side delivery rake, and chopped with a forage field chopper. All lots were dried on a slatted floor barn drier with supplemental heat. After drying, each lot of hay was baled and stored until fed.

Digestion trials were conducted on each lot of hay, using three milking cows in digestion stalls that permitted mechanical separation of the feces and urine. Ten-day preliminary periods and 10-day experimental periods were used for each lot of orchard grass. The animals were kept in the digestion stalls during the entire preliminary and experimental periods except for a daily period of

walking for exercise. The feeding schedule for the various stages of orchard grass hay fed is given in Table 1.

TABLE 1
Animals used and quantities of feed fed daily during the digestion trials.

Animal	Orchard grass hay	No. 86 grain
	(lb.)	*(lb.)*
X54 [a]	20	7
X65 [b]	25	5
2408	26	9
N635	16	7

[a] Used with stage 4 hay only.
[b] Not used with stage 4 hay.

The grain fed during the digestion trials was composed of 97 parts of ground corn, 2 parts of bone meal and 1 part of sodium chloride. Chromium oxide was added to the grain mixture by mixing 15 per cent chromium oxide with wheat flour and baking into a hard bread, which was dried and ground through a medium mesh screen in a Wiley mill and then mixed with each individual cow's ration in amounts that would provide daily intakes of approximately 15 g. of chromium oxide per cow.

During each 10-day experimental period, daily aliquots of feces samples were collected in duplicate as follows: (a) dried to constant weight in a circulating air sample drier for daily determinations of dry matter content and composited for the 10-day period for chemical analyses, (b) frozen daily and composited for the 10-day period for determination of the plant pigment concentration, and (c) composited in concentrated sulfuric acid for nitrogen determinations. Two samples of the hay fed daily were collected in duplicate as (a) a 200-g. sample dried to constant weight for determination of the dry matter content and composited for the 10-day period for chemical analysis, and (b) a daily sample composited as fed for determination of the plant pigment concentration.

In addition to the daily aliquots of the feces, "grab" samples were taken from two passages daily of each cow on three successive days during each 10-day experimental period. The "grab" samples were taken from a forenoon and afternoon passage of feces at approximately the time of day that had been shown to give representative concentrations of chromium and lignin in the feces during previous trials (28). Duplicate samples were dried for moisture determinations and chemical analysis, and duplicate samples were frozen as voided for determination of the plant pigment concentration, with the exception of the stage 4 orchard grass hay. Each duplicate daily "grab" sample was handled and analyzed as an individual sample designated as "first day partial," "second day partial," and "third day partial" for each stage of orchard grass hay fed. Corrections were made for the dry matter content of the "grab" sample to the total fecal dry matter excreted during the 10-day collection period.

Proximate analyses of the feed and feces were made according to the procedures described by the Association of Official Agricultural Chemists (4) for crude protein, ether extract, crude fiber, ash, and nitrogen-free extract.

Lignin was determined by the method of Ellis, Matrone, and Maynard (16).

The extraction of plant pigments in the feed and feces was made by the method of Reid *et al.* (37), but a unit was arbitrarily defined as that amount of plant pigment per milliliter of 85 per cent acetone extract that gave a density of 1.0 with a Beckmann model DU spectrophotometer when read in a 1-cm. cell. Absorption curves of the extracts from the feces and orchard grass hays were very similar to those reported by Reid *et al.* (37) for mixed hay and corresponding feces.

The method used for the determination of chromium oxide was a modification of the method of Edin *et al.* (15) and has been described in detail previously (27).

EXPERIMENTAL RESULTS AND DISCUSSION

The dry matter consumed in the grain fed each cow was the same for each digestion trial, but the dry matter consumption of the various stages of orchard grass hay varied slightly for the same animal because of slight variations in the dry matter content of the four stages of orchard grass hay. The chemical composition of the grain and the four stages of orchard grass hay fed each animal used in the digestion trials are presented in Table 2. Each analysis is the average of

TABLE 2

Composition of the dry matter of feeds used in digestion trials of orchard grass hay.

	Protein	Fiber	N.F.E.	Ether extract	Ash
	(%)	*(%)*	*(%)*	*(%)*	*(%)*
Grain 86	9.18	2.17	81.14	3.63	3.88
Orchard grass:					
Stage 1	24.78	26.88	35.02	3.99	9.33
Stage 2	15.80	28.15	45.82	3.46	6.77
Stage 3	13.00	31.77	44.24	3.91	7.08
Stage 4	12.38	35.05	41.16	4.24	7.17

at least four determinations secured by making replicate analyses on A and B samples of each material. The range in the stage of maturity represented by the four stages of orchard grass hay is indicated by the decrease in protein content from 24.78 to 12.38 per cent and the increase in the crude fiber content from 26.88 to 35.05 per cent with increasing maturity.

Digestion coefficients were calculated for the total collection samples, using the ratio technique with crude lignin, corrected lignin (crude lignin – N × 6.25), chromium oxide, and plant pigments. Digestion coefficients for the inert materials ratio techniques were calculated from the following formula:

$$\text{Digestion Coefficient} = 100 - \left(100 \times \frac{\%\ \text{inert material in feed}}{\%\ \text{inert material in feces}} \times \frac{\%\ \text{nutrient in feces}}{\%\ \text{nutrient in feed}}\right)$$

A summary of the various techniques of calculating the digestion coefficients is given in Table 3, using the average digestion coefficients of the three cows used with each stage of orchard grass hay. The digestion coefficients calculated

by the chromium ratio and the pigment ratio techniques on both the total collection samples and the 3-day partial collection samples compare very closely with the standard total collection technique for all of the constituents of the proximate analysis. Digestion coefficients calculated by the lignin ratio technique, using the crude lignin contents of the ration and feces, gave slightly lower digestion coefficients than were obtained by the total fecal collection technique. The digestion coefficients calculated by the corrected lignin ratio technique are slightly lower than those calculated by the crude lignin ratio technique.

A review of the literature on various digestion trial techniques indicates that previous comparisons of digestion trial techniques have not included a comparison of several methods of determining digestion coefficients in the same digestion trial. The present study has made a comparison of seven different methods of calculating digestion coefficients with each of four different stages of orchard grass hay fed with a uniform amount of grain.

Analysis of variance by the method of Snedecor (41) shows a significant difference between these seven methods of calculating digestion coefficients.

TABLE 3

Comparison of digestion coefficients calculated by several methods on four stages of orchard grass hay plus grain.

Constituent	Total collection samples [a]					Partial collection [b]	
	Standard	Chromium ratio	Lignin ratio		Pigment ratio	Chromium ratio	Pigment ratio
			Crude	Corrected			
Stage 1							
Dry Matter	73	74	71	68	72	74	69
Protein	67	69	66	63	67	70	65
Fiber	81	81	80	78	80	81	79
N.F.E.	79	78	76	74	77	77	73
Ether Ext.	47	48	43	38	44	50	41
Stage 2							
Dry Matter	74	75	71	69	75	75	77
Protein	63	65	59	56	65	69	71
Fiber	77	78	75	73	78	79	80
N.F.E.	80	81	78	77	81	80	81
Ether Ext.	46	48	40	36	48	48	51
Stage 3							
Dry Matter	69	67	66	65	67	70	70
Protein	59	56	55	53	56	62	62
Fiber	71	69	68	67	69	71	71
N.F.E.	76	75	74	73	75	76	76
Ether Ext.	40	35	34	31	36	36	37
Stage 4							
Dry Matter	66	66	61	60	64	67	....
Protein	59	60	55	54	58	63	....
Fiber	68	68	64	63	66	70	....
N.F.E.	72	71	67	66	69	71	....
Ether Ext.	39	39	30	29	35	41	....

[a] Av. for 3 cows.
[b] Av. for 3 daily samples (4 passages) for each of 3 cows.

There is a close check, however, between the digestion coefficients calculated by the standard total fecal collection, the chromium ratio, and the plant pigment ratio techniques. The results showed that the fecal recoveries of lignin are somewhat less than 100 per cent, which gave lower digestion coefficients calculated by the lignin ratio technique than those obtained by the total fecal collection technique. Further studies would be necessary to ascertain whether the digestibility of lignin in orchard grass hay is due to differences in the nature of the lignin isolated from the feed and feces studied; whether the metabolism of lignin in orchard grass hay is different than lignin metabolism in some other plants; or whether lignin metabolism of second growth orchard grass hay might be different than that of the first growth of the plant. Satisfactory results with the lignin ratio method of calculating digestion coefficients have been reported for other rations (16, 18, 19, 27, 42).

The dry matter digestibilities of the rations containing stage 1 and stage 2 hays were similar (Table 3). The slightly higher dry matter digestibility of the stage 2 hay compared with the stage 1 hay was accompanied by a decrease in the lignin content. The stage 2 hay presumably was cut during the period of the most rapid rate of growth of the grass. Huffman (23) has pointed out that the decreased digestibility of the dry matter of plants is believed to be associated with lignification of the cell wall, although the total amont of lignin does not appear to be the determining factor.

The apparent digestibility of the crude protein and crude fiber was higher in the ration containing stage 1 orchard grass than in the ration containing stage 2 orchard grass, but the digestibilities of the nitrogen-free extracts were similar. Digestibility of the dry matter, protein, crude fiber, and nitrogen-free extract fractions decreased progressively in the stage 2, stage 3, and stage 4 orchard grass hays.

SUMMARY

A comparison of seven different methods of determining digestibility coefficients was made with each of four digestion trials, using three milking cows fed orchard grass hay cut at four stages of maturity and a simple grain mixture. The conventional 10-day total fecal collection method was used as a standard for comparison with the results of the other techniques. Chromium oxide was added to the grain mixture as an exogenous inert substance. Lignin and certain plant pigments were determined as inert materials normally present in the ration. The concentrations of these three inert materials in the ration fed and in the fecal samples taken from an aliquot of the total collection were used in calculating the digestion coefficients by the inert material ratio technique. Additional "grab" samples taken on three consecutive days of the 10-day collection period were analyzed and the digestion coefficients calculated on each of these days by the chromium ratio and plant pigment ratio techniques. Incomplete recoveries of lignin with each of the total collection trials made it impractical to calculate the digestion coefficients by the lignin ratio technique with the "grab" samples.

Comparable digestion coefficients were secured with the standard 10-day

consumption-excretion method and the chromium and plant pigment ratio techniques when calculated with both the total collection samples and the averages of 3 days' partial collection or "grab" samples.

Digestion coefficients calculated by the crude lignin and the corrected lignin ratio techniques using the total fecal collection samples both were significantly lower than the digestion coefficients calculated by the standard consumption-excretion method in these trials.

Increasing maturity of the orchard grass hay resulted in a decrease in the protein content from 24.78 to 12.38 per cent and an increase in the crude fiber content from 26.88 to 35.05 per cent.

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A STUDY OF THE CRUDE FIBER AND NITROGEN-FREE EXTRACT FRACTIONS OF ORCHARD GRASS HAY AND THE DIGESTIBILITY OF SOME OF THE CONSTITUENTS BY MILKING COWS [1]

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Certain limitations of the widely used system of feedstuffs analysis inaugurated by Henneberg and Stohmann (22) undoubtedly have been recognized since the method was first proposed. The value and usefulness of this system of analysis also has been recognized and demonstrated through many years accumulation of results on a wide variety of feed materials. The extensive compilations of feed composition and digestibility data by Morrison (37) and Schneider (45) are a tribute to the past and present usefulness of the Henneberg system of feed analysis. The studies of Axelsson (3, 4, 5, 6, 7), Dijkstra (12), Duckworth (13), Hallsworth (20), Homb (24, 25), Jarl (26, 27), Lancaster (30), McMeekan (35), Nordfeldt *et al.* (38), and Olofsson (41) all indicate definite value in determining the crude fiber content of feeds since the high negative correlation between the crude fiber content and the digestibility and feeding value of a feed certainly justify the continued use of the crude fiber determination in the absence of a more desirable scheme of analysis.

There are numerous reports in the literature discussing the merits and limitations of partitioning the carbohydrates of feeds into crude fiber and nitrogen-free extract fractions. Several authors (8, 9, 10, 30, 32, 34, 36, 38, 39, 40, 50, 52) have criticized the Weende system of determining the content of crude fiber in a feed and calculating the amount of nitrogen-free extract as being unsatisfactory principally because the constituents that make up the crude fiber and nitrogen-free extract fractions vary greatly with different plant species and different materials and also with different methods of determining the crude fiber fraction.

Crampton and Maynard (10) have compared the average digestion coefficients of the nitrogen-free extract and crude fiber fractions in various classes of feeds. Dry roughages, pasture herbage, and silage had 39, 67, and 28 per cent, respectively, of the cases in which the crude fiber was as high or higher in digestibility than the nitrogen-free extract. This demonstrated the variability in the composition, digestibility, and feeding value of crude fiber from various sources.

Norman (39) pointed out that crude fiber determined by the conventional method was not a single compound but consisted of cellulose and lignin with some hemicellulose and pectin. Several modifications of the Weende system of analysis for crude fiber have been proposed but they all have the same criticism even though the reproducibility is satisfactory. Nordfelt *et al.* (38) compared

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several methods for the determination of crude fiber and found them to give good reproducibility, but different methods gave different values for crude fiber. They also showed that the composition of the crude fiber was different in different feeds and the crude fiber from a particular feed was different from that of the corresponding feces. They also demonstrated a selective digestibility of the lignin, cellulose, and pentosan fractions, which are the main components of the crude fiber. This work emphasized the value of lignin, cellulose, and pentosan determinations to supplement the crude fiber determination and thus give more useful information on the nature of the carbohydrate fraction in feeds and feces.

Knowledge of these limitations associated with the determination of crude fiber has stimulated interest in other systems of feedstuffs analysis that would not leave such a large portion of the feed analytically undetermined. A few investigators have attempted to account for nearly all the dry matter of various feeds and feces by analytical determinations which leave comparatively small amounts of undetermined substances.

Probably the earliest studies of the summative analysis of common feeding stuffs in the United States were made by Headden (21) and Widtsoe and Stewart (51). Headden (21) partitioned clover hay into sugars, dextrins, starch, xylans, lignones, cellulose, moisture, ash, proteins, materials extracted by ether, and materials extracted by alcohol and water, corrected for sugar and dextrins. He had 4.8 per cent of the total plant material undetermined by this system of analysis. Widtsoe and Stewart (51) partitioned the dry matter of alfalfa into the ether extract, water extract, acid extract, alkali extract, fiber, and ash in the fiber fractions. They made progressive extractions with each solvent on the residue remaining after previous extractions, thus accounting for all of the dry matter. This system would not be too useful for digestibility studies, since the material extracted by each solvent from the feed and feces probably would vary widely.

Crampton and Whiting (11) proposed a scheme of analysis which determined the percentage of lignin by difference after making analytical determinations of cellulose and soluble carbohydrates. Ferguson (15) analyzed wheat straw for its content of ether extract, crude protein, ash, lignin, true cellulose, pentosans in cellulose and furfuraldehyde. With this system of analysis he had 13.7 per cent of the dry matter undetermined, which he called "other carbohydrates." Ferguson (16) also analyzed several forage samples for the individual structural constituents of the cell wall and also for the non structural constituents and secured variable quantities of undetermined constituents. Analysis of very young high protein grass gave undetermined fractions amounting to only 4.5 per cent of the dry matter while the more mature forages had over 16 per cent of the dry matter undetermined. Reid (43) reported that larger amounts of the dry matter of mature grass than of younger grasses were undetermined by the sum of the lignin, cellulose, hemicellulose, pectin, starch, total sugar, ash, crude protein and ether extract percentages.

Flanders (17) recently reported a summative analysis of timothy hay by determining ash, benzene-alcohol extractives, water extractives, pectic substances,

hemicelluloses, alpha cellulose, lignin, and proteins. A study of the four hemicellulose fractions extracted indicated that they were mixtures and their composition varied from one plant source to another (18). The hemicellulose fractions showed comparable variations in the uronic acid anhydride and pentose contents within plant families but not between plants of different families.

The present status of our knowledge of the carbohydrate constituents of forage has been well described by Percival (42) in a recent discussion of the subject. He stated that: "Although an immense amount of routine analytical work has been carried out on forage crops in the past, we are still ignorant, in the main, of what is measured under such headings as crude fiber, total carbohydrates, and so on."

It seemed desirable to study certain of the chemical constituents of the crude fiber and nitrogen-free extract fractions that could be determined as either definite chemical substances or that would be representative of similar substances when determinations are made on widely different materials, such as feeds and feces. Although such a method of analysis is not readily applicable to routine determinations, it would give useful information on the relative digestibilities of various fractions of the carbohydrate complex of forage plants and also might permit logical grouping of certain of these constituents that would be biologically similar and might be grouped easily for convenient chemical determinations.

The object of this investigation was to determine the digestion coefficients of certain constituents that ordinarily are grouped in the crude fiber and nitrogen-free extract fractions and also to ascertain what percentage of the dry matter of the feed and feces would be accounted for by a more detailed system of analysis.

EXPERIMENTAL PROCEDURE

The method of conducting the digestion trials and the collection and handling of the samples has been described previously (29).

Cellulose was determined by the method of Matrone, Ellis, and Maynard (33).

Pentosans were determined as the furfural-yielding constituents, according to the official method of the Association of Official Agricultural Chemists (1), and the weight of furfural phloroglucide was calculated as the pentosan equivalent. The pentosan content of the Matrone crude cellulose was determined on a sample of crude cellulose which was divided into separate samples for determinations of the pentosan and cellulose contents.

Starch was determined on the grain samples by the official diastase method with subsequent acid hydrolysis of the Association of Official Agricultural Chemists (1). Starch was determined on the hay and feces samples by the method of Hoffpauir (23).

Determinations of the reducing sugars were made by extracting the material with 80-per cent alcohol according to the method of Thomas, Melin, and Moore (49), inversion with hydrochloric acid, and determining the reduced copper after boiling the extract with copper sulfate-alkaline tartrate solution by the dichromate-ferric sulfate-diphenylamine procedure described by Leonard *et al.* (31).

Organic acids were determined by the method of Somers (46). Aqueous extractions of the material were made in a Waring blender after addition of sufficient 3 *N* hydrochloric acid to bring the pH to approximately 1.0. The acidified extracts were heated in a steam bath to remove carbon dioxide, made to a convenient volume at 25° C., and then a 50-ml. aliquot was adjusted to pH 7.8 with sodium hydroxide (3 *N* followed by 0.05 *N* sodium hydroxide) and titrated to pH 2.6 with 0.05 *N* nitric acid. The organic acids were calculated as the equivalent amount of malic acid.

Alpha cellulose was determined as that portion of the Matrone crude cellulose that was insoluble in 17.5 *N* sodium hydroxide, according to the method described by the Technical Association of the Pulp and Paper Industry (47).

RESULTS AND DISCUSSION

The composition of the crude fiber and nitrogen-free extract fractions of the feeds fed during the digestion trials conducted on the orchard grass hay cut at four stages of maturity are given in Table 1. The conventional proximate system

TABLE 1
Composition of the feeds used for digestion trials

	Orchard grass hay				No. 86 grain
	Stage 1	Stage 2	Stage 3	Stage 4	
Protein	24.8	15.8	13.0	12.4	9.2
Ash	9.3	6.8	7.1	7.2	3.9
Ether extract	4.0	3.5	3.9	4.2	3.6
Sugars	2.1	9.5	5.4	2.4	1.4
Starch	1.2	1.4	0.8	0.9	65.0
Organic acids	6.3	6.0	5.4	5.0	...
Pentosans	15.1	15.8	16.8	18.1	6.4
Alpha cellulose	19.5	19.8	19.1	27.7	2.3
Beta and gamma cellulose	3.4	5.4	3.8	2.5	...
Lignin	5.7	5.0	6.2	8.1	0.5
Undetermined	8.6	11.0	18.5	11.5	7.7
Crude fiber	26.9	28.2	31.8	35.0	2.2
N.F.E.	35.0	45.7	44.2	41.2	81.1
Total carbohydrates [a]	49.9	63.0	64.4	63.1	82.8
Holocellulose [b]	46.5	52.0	58.2	59.8	16.4

[a] Total carbohydrates = (crude fiber + N.F.E.) — (lignin + organic acids).
[b] Holocellulose = total carbohydrates — (starch + sugar).

of analysis for feeds includes determinations of the protein, ash, ether extract, and crude fiber percentages and calculation of the nitrogen-free extract fraction of the feed by difference. With this system, the percentages that were determined by difference (nitrogen-free extract) ranged from 35.0 to 45.7 per cent of the dry matter of the hays. When analyses were made of the sugar, starch, organic acid, pentosan, alpha cellulose, and lignin contents of the hay, the undetermined portion of the total hay ranged from 8.6 to 18.5 per cent of the dry matter. These percentages of the undetermined portion of the dry matter of feeds are within the same range as reported by Ferguson (15, 16). Reid (43) accounted for somewhat higher percentages of the total dry matter of forages.

A greater percentage of the dry matter of the immature orchard grass hay was accounted for by the more detailed system of analysis than in the more mature orchard grass hay used in this study. This trend has been reported also by Reid (43). Differences in the per cent of dry matter accounted for in the immature and mature forages by this system of analysis may be due partially to the increase in the proportion of the dry matter of the hay which the crude fiber and nitrogen-free extract fractions represent with increasing maturity. The total of these two fractions increased from 61.9 per cent in the immature hay to 76.2 per cent in the mature hay. Errors due to conversion of the nitrogen content of young plants to protein equivalent also may be greater in young plants than in older plants. It is recognized also that the undetermined fraction includes any analytical errors involved in each of the individual analyses. With the proximate system of analysis these errors are included in the nitrogen-free extract fraction.

Several authors (2, 19, 28, 44, 48, 53) have given summative analyses of woods that are remarkably close to 100 per cent of the original material. Most of these experiments have included the total carbohydrate fraction of woods in preparations of holocellulose. There is evidence that properly isolated holocellulose retains nearly all of the pentosans, acetyl groups, and the carbon dioxide-forming material originally present in the wood (44). Another approach to the summative analysis of forages would be to include several of the carbohydrate fractions present in forages in a single determination of related materials such as those included in holocellulose preparations. The feasibility of such a system would depend upon suitable techniques for isolating the holocellulose fraction from forages and would necessitate also a knowledge of the digestibility of the various constituents of the holocellulose fraction.

The lignin, total cellulose, and pentosan content of the immature orchard grass hay was lower than that of the mature orchard grass hay. The organic acid content of the orchard grass hay decreased with advancing maturity. The sugar content of the most immature orchard grass hay was lower than in the hays cut at later stages of maturity. The sugar content of the hays cut at the three later stages of maturity progressively decreased with advancing maturity. The low sugar content of the immature hay probably is due to its being placed on the barn dryer at a higher moisture content than the other stages, thus permitting plant cell respiration to continue for a longer period than in the three other stages, which were partially dried in the field before being placed on the barn dryer.

The detailed composition of the crude fiber and nitrogen-free extract fractions of the feces of each animal used in the digestion trials is shown in Table 2. The nitrogen-free extract in these feces ranged from 35.7 to 44.6 per cent. When a more detailed system of analysis was used, including the determinations of sugars, starch, organic acids, pentosans, alpha cellulose, beta and gamma cellulose, and lignin, the undetermined portion of the feces dry matter ranged from 0.1 to 9.6 per cent. The more detailed system of analysis accounted for a higher percentage of the dry matter of fecal material than of the hays.

TABLE 2

Composition of the feces of cows fed orchard grass hay cut at four stages of maturity.

Constituent	Stage 1			Stage 2			Stage 3			Stage 4		
	X65	2408	N635	X65	2408	N635	X65	2408	N635	X54	2408	N635
Protein	24.9	26.5	27.0	20.2	20.3	19.6	15.7	16.8	15.5	12.6	14.3	13.5
Ash	15.7	14.8	14.9	13.7	12.5	13.2	11.4	12.2	12.3	11.1	11.0	10.9
Ether extract	8.1	7.3	7.7	7.6	6.8	7.4	7.9	7.2	7.5	7.4	7.1	6.8
Sugars	0.5	0.3	0.2	0.2	0.5	0.5	0.3	0.2	0.3	0.4	0.4	0.3
Starch	1.2	3.8	2.0	2.1	3.7	2.7	1.2	3.6	2.3	1.6	3.0	2.0
Organic acids	8.3	8.4	8.2	7.1	7.4	8.3	6.5	6.8	6.9	5.9	5.8	5.9
Pentosans	10.5	10.5	10.5	14.2	13.3	13.7	16.1	15.7	15.8	16.9	16.1	16.6
Alpha cellulose	11.5	11.5	10.2	13.4	13.9	12.8	15.3	15.4	15.2	17.2	16.5	17.4
Beta and gamma cellulose	2.5	2.5	3.0	3.9	1.8	5.0	3.2	3.1	3.3	1.6	4.0	3.2
Lignin	15.4	14.3	14.4	14.2	12.7	13.6	15.4	13.6	14.0	15.7	15.0	15.6
Undetermined	1.4	0.1	1.9	3.4	7.1	3.2	7.0	5.4	6.9	9.6	6.8	7.8
Crude fiber	15.6	14.3	14.7	20.3	18.9	18.4	24.5	22.7	22.9	24.8	23.8	24.2
N.F.E.	35.7	37.1	35.7	38.2	41.5	41.4	40.5	41.1	41.8	44.1	43.8	44.6
Total carbohydrates [a]	27.6	28.7	27.8	37.2	40.3	37.9	43.1	43.4	43.8	47.3	46.8	47.3
Holocellulose [b]	25.9	24.6	25.6	34.9	36.1	34.7	41.6	39.6	41.2	45.3	43.4	45.0

[a] Total carbohydrates = (crude fiber + N.F.E.) — (lignin + organic acids).

[b] Holocellulose = total carbohydrates — (starch + sugar).

When orchard grass hays of increasing maturity were fed, the alpha cellulose, pentosan, and lignin contents of the feces increased. The increase in the lignin content of the feces is small compared to the change in the lignin content of the hays, since decreases in the digestibility of the total dry matter reduce this change.

The content of reducing sugars was very low in all of the feces. The starch content of the feces ranged from 1.2 to 3.8 per cent and showed about the same range of values for the three cows when fed each of the four orchard grass hays.

The cellulose aggregate isolated from plant material by the Matrone, Ellis, and Maynard (33) method includes a large part of the hemicellulose and related materials which are furfural-yielding constituents and are included in the pentosan fraction in this study. This does not permit a summative analysis of the forage to be made using the crude cellulose, since it would duplicate a portion of the pentosan fraction in the crude cellulose fraction. Determinations of the pentosan content of crude cellulose isolated by the method of Matrone, Ellis, and Maynard (33) were made in order to calculate a "corrected Matrone cellulose,"

TABLE 3

Matrone crude cellulose content of orchard grass hays and the corresponding feces and the distribution of its various constituents

	Constituents of Matrone cellulose				
	Matrone crude cellulose [a]	Alpha cellulose [b]	Pentosans [b]	Beta and gamma cellulose [b]	Corrected Matrone cellulose [c]
	%	%	%	%	%
O.G. Hay					
Stage 1	34.0	62.6	26.3	11.1	25.1
Stage 2	34.7	61.0	28.6	10.4	24.8
Stage 3	37.7	61.7	25.8	12.5	28.0
Stage 4	40.3	67.5	26.4	6.1	29.7
Feces					
Stage 1					
X65	17.8	61.4	25.1	13.5	13.3
2408	17.8	61.4	25.1	13.5	13.3
N635	16.8	58.4	24.4	17.2	12.7
Stage 2					
X65	23.9	57.9	25.4	16.7	17.8
2408	22.2	64.2	27.4	8.4	16.1
N635	21.8	55.5	22.6	21.9	16.9
Stage 3					
X65	26.6	61.6	25.4	13.0	18.8
2408	26.2	61.7	25.7	12.6	19.5
N635	27.1	61.6	25.2	13.2	20.3
Stage 4					
X54	27.7	67.1	26.8	6.1	20.3
2408	27.1	63.5	24.2	12.3	20.5
N635	27.2	60.8	24.7	14.5	20.5

[a] Per cent of constituent in feed or feces dry matter.
[b] Per cent of constituent in Matrone crude cellulose.
[c] Corrected Matrone cellulose = Matrone crude cellulose — pentosans.

which was free of pentosans and would not cause duplication in a system of summative analysis which also included the pentosan fraction. Alpha cellulose was determined on separate samples of crude cellulose, and the remainder of the crude cellulose which was not determined as alpha cellulose and pentosans was calculated as beta and gamma cellulose. The data are shown in Table 3. Apparently the proportions of alpha cellulose and pentosans in the cellulose material isolated by the Matrone, Ellis, and Maynard (33) method does not change appreciably with increasing maturity. Furthermore, the ratio of alpha cellulose to pentosans in the Matrone crude cellulose isolated from the feces does not differ greatly from that isolated from orchard grass hay. The fraction designated as the "beta and gamma cellulose" was calculated as the residue from the Matrone crude cellulose that was not determined as alpha cellulose or pentosans. This fraction is quite variable in the amount present in various materials.

The content of "corrected Matrone cellulose" follows the same trends in the hay and the corresponding feces as the Matrone crude cellulose.

The apparent digestion coefficients of the various constituents of the carbohydrate fraction and related constituents are given in Table 4. It is evident that the apparent digestion coefficients of the Matrone crude cellulose, corrected Matrone cellulose, alpha cellulose, holocellulose, pentosans, total carbohydrate,

TABLE 4

Apparent digestion coefficients of carbohydrates and related constituents in orchard grass hay cut at four stages of maturity

	Crude cellulose	Corrected cellulose	Alpha cellulose	Holocellulose	Pentosans	Starch	Sugar	Total carbohydrates	Organic Acids	Undetermined fraction
Stage 1										
X65	82	82	80	82	78	97	96	86	55	95
2408	82	81	80	83	79	94	97	87	52	99
N635	83	82	82	83	79	98	94	88	53	94
Av.	82	82	81	83	79	96	96	87	53	96
Stage 2										
X65	77	76	78	79	72	95	99	84	60	91
2408	79	78	77	79	75	95	98	85	58	82
N635	79	77	78	79	74	97	98	86	52	92
Av.	78	77	78	79	74	96	98	85	57	88
Stage 3										
X65	72	73	69	73	64	97	98	79	52	85
2408	72	72	68	75	66	93	99	81	49	90
N635	72	71	69	74	67	97	98	82	48	87
Av.	72	72	69	74	66	96	98	81	50	87
Stage 4										
X54	70	70	73	69	64	97	95	77	46	69
2408	69	68	73	69	63	94	93	76	45	77
N635	69	68	71	68	62	97	95	77	44	75
Av.	69	69	72	69	63	96	94	77	45	74

organic acids, and undetermined fractions all decreased with advancing maturity of the orchard grass hay. The digestibility coefficients of the starch and sugar fractions were high with all of the four stages of maturity. A comparison of the apparent digestion coefficients of the Matrone crude cellulose and the corrected Matrone cellulose would indicate that there is very little difference in the digestibility of the alpha cellulose and pentosan fractions of the Matrone crude cellulose. A comparison of the apparent digestion coefficients of the total pentosan fraction of the feed and the Matrone crude cellulose indicates that the pentosans not associated with the Matrone crude cellulose had a somewhat lower digestion coefficient than the pentosans included with the Matrone crude cellulose.

The apparent digestion coefficients of the undetermined fractions were calculated by assuming that these fractions were of the same composition in the feed and feces. The digestibility of the undetermined fraction of the immature forage indicated that this fraction is as highly digested as the starch and sugar. The digestibility of this fraction decreased with increasing maturity and was digested to about the same extent as the alpha cellulose in the most mature stage of orchard grass.

A comparison of the digestion coefficients of the corrected Matrone cellulose, alpha cellulose, holocellulose, and pentosan fractions of any stage of maturity of orchard grass hay would indicate that the digestibility of these groups is similar enough to justify grouping them into one fraction of similar substances, such as might be accomplished in a holocellulose determination. The object of a more detailed analysis of the carbohydrate fraction is not to increase the analytical determinations necessary but to allow intelligent grouping of various constituents into groups of substances that would be similar from an analytical, biological, and chemical standpoint.

SUMMARY

A detailed system of analysis that included determinations of the starch, sugars, organic acids, pentosans, alpha cellulose, and lignin was used with orchard grass hay cut at four stages of maturity and with the corresponding feces to study the constituents ordinarily grouped in the crude fiber and nitrogen-free extract fractions.

A greater percentage of the dry matter of the immature orchard grass hay was accounted for by this system of analysis than of the more mature orchard grass hay used in this study.

Higher percentages of the dry matter were accounted for by the detailed system of analysis in the feces of animals fed the immature orchard grass hay than in the feces from animals fed the mature orchard grass hay.

Considerably higher percentages of the total dry matter of feces were determined by this system of analysis than were determined with the same analytical techniques on the forage fed to these animals.

Cellulose isolated from the orchard grass hays cut at four stages of maturity and from the corresponding feces by the method of Matrone, Ellis, and Maynard contained approximately 25 per cent of furfural-yielding constituents calculated

as pentosans. The apparent digestion coefficients of the cellulose corrected for pentosans was not appreciably different from the digestion coefficients of the crude cellulose fraction.

The apparent digestion coefficients of the Matrone crude cellulose, corrected Matrone cellulose, alpha cellulose, pentosans, total carbohydrates, organic acids, and undetermined fractions all decreased with advancing maturity of the orchard grass hay. The digestion coefficients of the starch and sugar fractions of the orchard grass hay were high with all of the four stages of maturity.

The similarity in the digestion coefficients of the alpha cellulose, holocellulose, pentosan, and Matrone crude cellulose fractions indicate that a large part of the forage that is ordinarily included in the crude fiber and nitrogen-free extract fractions could justifiably be grouped into a holocellulose fraction that could be analytically determined.

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STUDIES ON THE COMPOSITION OF LIGNIN ISOLATED FROM ORCHARD GRASS HAY CUT AT FOUR STAGES OF MATURITY AND FROM THE CORRESPONDING FECES [1]

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Lignin has been reported by a number of workers to be indigestible (7, 9, 13, 14, 16, 17, 20, 39). Other reports indicate that varying amounts of lignin may be digested or broken down in the ruminant digestive tract (4, 8, 10, 11, 18, 22, 23, 28).

Since the chemical structure of lignin has not been established, it is not feasible to isolate or determine a product of uniform composition from different materials. Certain characteristics of crude lignin preparations vary considerably, depending upon the material from which it is isolated and the method used for its isolation. Extensive studies have been made of the characteristics of lignin isolated from various woods, but only limited studies have been reported on lignin prepared from forage plants important in animal feeding.

Phillips *et al.* (34) found 4.34 per cent nitrogen in the lignin obtained from oat plants 7 days old, which decreased to 1.26 per cent nitrogen in the lignin from plants 84 days old. Bondi and Meyer (4) reported from 1.18 to 1.63 per cent nitrogen in the lignin from four grass species and from 2.92 to 3.36 per cent nitrogen in the lignin from four legumes. The lignin isolated from the corresponding feces had slightly higher nitrogen contents. They also showed that the lignin nitrogen was resistant to hydrolysis by strong acid (5 *N* H_2SO_4), by weak acid (0.5 *N* HCl), and by pepsin added to the weak acid. These results led these investigators to conclude that the nitrogen in crude lignin preparations was not present as protein nitrogen. Furthermore, the digestive enzymes of the ruminant did not reduce the nitrogen content of the lignin.

Norman and Jenkins (26) believed that protein degradation products following the treatment of plant material with strong acid resulted in high lignin yields rather than protein *per se*. Paloheimo (27), Waksman and Stevens (41), Phillips (29), and DeMan and DeHeus (12) have corrected crude lignin values by deducting the nitrogen content multiplied by the factor 6.25. Phillips (30) later felt that, because there was not a constant relation between the amount of protein added to samples for the determination of lignin and the size of the error involved, the apparent lignin values should not be corrected but the per cent of nitrogen in the lignin should be reported.

Thomas and Armstrong (40) found amino acids in the hydrolysates from crude lignin and proposed that the factor 6.25 for converting lignin nitrogen to protein was valid. DeMan and DeHeus (12) found the tyrosine and the tryptophane nitrogen in the lignin isolated from Lolium perenne was in agree-

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[1] From a portion of a thesis presented by the senior author to the Graduate School of Michigan State College in partial fulfillment of the requirements for the Ph.D. degree.

ment with that for the true protein of the grass. The tryptophane nitrogen was determined on lignin isolated without the use of concentrated sulfuric acid.

Bondi and Meyer (4) found about 5 per cent methoxyl in the lignin obtained from legumes and about 10 per cent methoxyl in the lignin isolated from various grasses. They also found very little difference in the methoxyl content of the lignin isolated from the corresponding feces, which would indicate that there is very little breakdown of the ether linkages in the lignin molecule during passage through the digestive tract. Oxidation of the lignin from various plants gave yields of 4.2 to 21.6 per cent aldehydes, but oxidation of the fecal lignins gave negligible quantities of aldehydes, indicating a change in the side chains attached to the lignin molecule during passage through the intestinal tract. The lignin from the grasses gave a much higher percentage of aldehydes following oxidation than did that from legumes, but the ratio of vanillin to p-hydroxybenzaldehyde was approximately 2 to 1 in all cases.

Stone, Blundell, and Tanner (38) found that the ratios of vanillin to syringealdehyde did not remain constant in the wheat plant at different stages of growth since the percentage of syringealdehyde was lower than vanillin in the young plants and higher in the more mature plants. Concurrent increases in the methoxyl content of the lignin were observed.

Csonka, Phillips, and Jones (10) and Phillips *et al.* (35) reported a loss of 36.7 per cent of the methoxyl groups associated with the lignin in the digestive tract of a cow fed a ration of hay, grain, and silage. Francois, Leroy, and Levy (15) reported that 50 per cent of the methoxyl content of the lignin disappeared in the digestive tract of ruminants fed alfalfa hay alone and in combination with wheat, and dried beet pulp. The apparent digestibility of the lignin of alfalfa was approximately 8 per cent.

Pazur and DeLong (27), Phillips *et al.* (35) and Csonka, Phillips, and Jones (10) reported increased urinary excretion of hippuric acid when increased amounts of lignin are fed and believed that this indicated a metabolism of lignin in the animal body. Rogozinski and Starzewska (37) believed that lignin was indigestible by sheep and played no part in the formation of hippuric acid.

This study was conducted to determine certain characteristics of the lignin isolated from orchard grass hay cut at four stages of maturity and from the corresponding feces of dairy cows fed these forages.

EXPERIMENTAL PROCEDURE

The method of conducting the digestion trials and the collection and handling of the samples have been presented previously (19).

The nitrogen content of the lignin was determined by the microkjeldahl method of the Association of Official Agricultural Chemists (3) on samples of the lignin isolated from the various materials by the method of Ellis, Matrone, and Maynard (13).

The total methoxyl groups in the feeds fed and the feces voided and also the methoxyl content of the lignin isolated from these materials were determined by the method of Clark (5, 6).

The benzoic acid concentration in the urine was determined by the method of Kingsbury and Swanson (21).

RESULTS AND DISCUSSION

The amounts of lignin consumed and excreted and the per cent recovery of lignin in the feces and the per cent of lignin digested are given in Table 1. The

TABLE 1

Crude lignin fed and excreted by each animal receiving orchard grass hay harvested at various stages plus grain

Hay fed	Animal	Average daily crude lignin consumed			Average daily lignin excreted	Lignin recovery	Lignin digested
		Hay	Grain	Total			
		(g.)	*(g.)*	*(g.)*	*(g.)*	*(%)*	*(%)*
Stage 1	X65	781.0	10.3	791.3	741.8	93.7	6.3
	2408	812.3	18.4	830.7	763.9	92.0	8.0
	N635	499.8	14.4	514.2	479.7	93.3	6.7
Stage 2	X65	695.6	10.3	705.9	660.8	93.6	6.4
	2408	723.4	18.4	741.8	623.2	84.0	16.0
	N635	445.2	14.4	459.6	408.7	88.9	11.1
Stage 3	X65	816.3	10.3	826.6	795.4	96.2	3.8
	2408	848.9	18.4	867.3	754.8	87.0	13.0
	N635	532.4	14.4	536.8	480.0	89.4	10.6
Stage 4	X54	696.9	15.5	712.4	624.5	87.7	12.3
	2408	1006.6	18.4	1025.0	886.0	86.4	13.6
	N635	619.4	14.4	633.8	578.2	91.2	8.8

lignin recoveries all were somewhat lower than is desirable for use as an inert tracer material. This finding explains the tendency for the lower digestion coefficients previously presented (19) that were calculated by the lignin ratio technique. When planning this experiment, it was postulated that, if the lignin of orchard grass was partially digested, the lignin of immature orchard grass might be more digestible than that of orchard grass of the more mature stages. Since lower digestibility of the lignin in the immature orchard grass hay ration was secured, it seemed desirable to investigate some of the characteristics of the lignin isolated from the feeds and feces from these digestion trials. The nitrogen content of the lignin isolated from the four stages of orchard grass hay and the corresponding feces is presented in Table 2. The lignin isolated from the immature orchard grass had a higher nitrogen content (4.25 per cent) than the lignin isolated from the most mature orchard grass (2.41 per cent). Lignin isolated from the corresponding feces had average nitrogen contents of 5.19 and 2.19 per cent, respectively. The protein content of the dry matter of the immature grass was 24.78 per cent and the protein content of the most mature orchard grass was 12.38 per cent. The protein content of the corresponding feces averaged 26.09 and 13.83 per cent, respectively. Small differences were obtained between the nitrogen content of the lignin from each stage of orchard grass hay and the

TABLE 2

Nitrogen content of the crude lignin isolated from orchard grass hay of various stages of maturity and the corresponding feces

Stage of maturity	Material	Animal	Crude lignin	Nitrogen	True [a] lignin
			(%)	*(%)*	*(%)*
Stage 1	Hay	.....	7.77	4.25	5.71
	Feces	X65	21.61	5.07	14.77
		2408	20.20	5.16	13.69
		N635	20.63	5.34	13.74
Stage 2	Hay	.....	6.74	4.15	5.00
	Feces	X65	19.04	4.35	13.23
		2408	16.99	4.07	12.11
		N635	17.63	3.98	12.71
Stage 3	Hay	.....	7.97	3.53	6.21
	Feces	X65	19.32	3.49	14.92
		2408	17.36	3.69	13.18
		N635	18.12	3.67	13.36
Stage 4	Hay	.....	9.66	2.41	8.12
	Feces	X54	18.83	3.18	15.09
		2408	17.88	2.69	14.72
		N635	18.64	2.85	15.25

[a] Crude lignin minus (Nitrogen × 6.25).

nitrogen content of the lignin isolated from the corresponding feces. The "true lignin" contents of the hays at each stage of maturity and the corresponding feces were calculated by deducting from the crude lignin percentage the nitrogen contained in the crude lignin multiplied by the factor 6.25. Although there is considerable controversy concerning the nature of the nitrogen present in the lignin preparations, the work of DeMan and DeHeus (12) and Thomas and Armstrong (40) indicates that the use of a protein factor may be justified in converting the nitrogen present in the lignin to protein.

The crude lignin contents of the four stages of orchard grass hay of increasing maturity were 7.77, 6.74, 7.97, and 9.56 per cent, respectively. Lignin is generally considered as a constituent that increases with advancing maturity of the plant (2, 9, 24, 31, 32, 33, 34, 36, 42). Other work (2, 25) with orchard grass has shown that the lignin content initially decreases and then progressively increases with advancing maturity, and these changes are concomitant with progressive increases in the crude fiber content and progressive decreases in the crude protein content. These results are in agreement with those found in this investigation. There is evidence that similar changes occur in timothy (32), oats (34), perennial ryegrass (2), tall fescue (2), and burnet (2). This pattern of change has not been observed in alfalfa, trefoil, and yarrow (2). Whether these changes are a physiological characteristic of growth of certain grasses or whether there is an inherent error in the determination of lignin in immature grasses has not been determined.

TABLE 3

True lignin fed and excreted by each animal receiving orchard grass cut at various stages plus grain

Hay Fed	Animal	Average daily true lignin consumed			Average daily lignin excreted	Lignin recovery	Lignin digested
		Hay	Grain	Total			
		(g.)	*(g.)*	*(g.)*	*(g.)*	*(%)*	*(%)*
Stage 1	X65	573.4	10.0	583.4	507.0	86.9	13.1
	2408	596.4	18.0	614.4	517.7	84.3	15.7
	N635	367.0	14.1	381.1	319.5	83.8	16.2
Stage 2	X65	515.3	10.0	525.3	459.2	87.4	12.6
	2408	535.9	18.0	553.9	444.2	80.2	19.8
	N635	329.8	14.1	343.9	294.7	85.7	14.3
Stage 3	X65	636.3	10.0	646.3	597.8	92.5	7.5
	2408	661.7	18.0	679.7	573.1	84.3	15.7
	N635	407.2	14.1	421.3	353.9	84.0	16.0
Stage 4	X54	592.0	15.2	607.2	500.4	82.4	17.6
	2408	855.1	18.0	873.1	729.4	83.5	16.5
	N635	526.2	14.1	540.3	474.1	87.8	12.2

The true lignin intakes and excretion for each stage of hay fed were calculated, and these data with the recoveries and digestibilities of the true lignin are presented in Table 3. The apparent digestion coefficients for true lignin are higher than those calculated for crude lignin. If the recoveries of the true lignin were higher than those found for the crude lignin, one could assume that a higher digestibility of the nitrogen containing fraction of the crude lignin had resulted in greater losses of this portion in passing through the digestive tract. Comparison of the nitrogen contents of the lignin isolated from the various

TABLE 4

Urinary excretion of benzoic acid by animals fed orchard grass hay cut at four stages of maturity

Stage	Animal	Average daily crude lignin		Average daily true lignin		Benzoic acid excretion				
						Total daily	g./100 g. Crude lignin		g./100 g. True lignin	
		Fed	Digested	Fed	Digested		Fed	Digested	Fed	Digested
		(g.)	*(g.)*	*(g.)*	*(g.)*	*(g.)*				
1	X65	791	49.5	583	76.4	139	17.6	280.8	23.8	181.9
	2408	831	66.8	614	96.7	115	13.8	172.2	18.7	118.9
	N635	514	34.5	381	61.6	80	15.6	231.9	21.0	129.9
2	X65	706	45.1	525	66.1	136	19.3	301.6	25.9	205.7
	2408	742	118.6	554	109.7	156	21.0	131.5	28.2	142.2
	N635	460	50.9	344	49.2	115	25.0	225.9	33.4	233.7
3	X65	827	31.2	646	48.5	122	14.8	391.0	18.9	251.5
	2408	867	112.5	680	106.6	118	13.6	104.9	17.4	110.7
	N635	537	56.8	421	67.4	97	18.1	170.8	23.0	143.9
4	X54	712	87.9	607	106.8	104	14.6	118.3	17.1	97.4
	2408	1025	139.0	873	143.7	92	9.0	66.2	10.5	64.0
	N635	634	55.6	540	66.2	61	9.6	109.7	11.3	92.1

orchard grass hays and the corresponding feces (Table 2) does not indicate that this occurred.

Since other studies (10, 28, 35) have indicated that degradation products of lignin may cause increased urinary excretion of benzoic acid, determinations were made of the benzoic acid excretion by each animal fed the orchard grass hay harvested at four stages. Table 4 gives the amounts of crude lignin and true lignin fed and digested by each animal and the urinary excretion of benzoic acid per 100 g. of lignin fed and digested. The benzoic acid excretion per unit of lignin fed is somewhat lower in the mature hay than in less mature hays harvested at the three other stages. If the amount of urinary benzoic acid excreted is influenced largely by the degree of lignin degradation, these figures would indicate a lower digestibility of lignin in the more mature orchard grass hay. This is not supported by the consumption-excretion figures for crude lignin given in Table 1. It is possible that other constituents of immature orchard grass contribute to the formation and excretion of benzoic acid.

The methoxyl contents of the feed and feces on a dry-weight basis are given in Table 5. A progressive increase in the total methoxyl content of the hay occurs with increasing maturity from 1.41 per cent to 1.85 per cent, and similar increases occur in the methoxyl content of the feces from animals fed these hays.

TABLE 5

Methoxyl content of the feed and feces dry matter and the lignin isolated from these materials, and digestion coefficients for various fractions of methoxyl groups

Stage	Material	Animal	Total methoxyl	Lignin methoxyl	Lignin methoxyl in total	Digestion coefficients		
						Total methoxyl	Lignin methoxyl	Non-Lignin methoxyl
			(%)	*(%)*	*(%)*			
1	Hay		1.41	7.84	31.9			
	Feces	X65	1.84	7.75	63.6	58.4	16.0	77.3
		2408	1.77	7.39	59.3	59.7	23.5	75.4
		N635	1.72	7.70	63.4	61.0	21.5	79.2
		Ave.	1.78	7.61	62.1	59.7	20.3	77.3
2	Hay		1.51	10.60	35.1			
	Feces	X65	2.09	8.80	58.4	56.4	28.4	70.8
		2408	2.08	8.78	53.4	57.8	35.1	69.1
		N635	2.08	8.88	56.7	57.9	31.3	71.3
		Ave.	2.08	8.82	56.2	57.4	31.6	70.4
3	Hay		1.62	10.73	40.7			
	Feces	X65	2.25	9.30	62.2	47.5	20.7	65.2
		2408	2.08	8.68	57.2	52.7	33.0	65.3
		N635	2.40	9.52	55.4	47.4	27.2	59.9
		Ave.	2.24	9.17	58.3	49.2	27.0	63.5
4	Hay		1.85	10.41	45.4			
	Feces	2408	2.35	9.77	60.9	45.5	22.8	62.8
		N635	2.50	9.96	59.6	42.4	17.7	60.6
		Ave.	2.43	9.87	60.3	44.0	20.3	61.7

The methoxyl content of the lignin from the immature hay is lower (7.84 per cent) than that from the three other stages, which are similar in methoxyl content (10.60, 10.73, and 10.41 per cent). Comparable changes occur in the methoxyl content of the lignin isolated from the corresponding feces. Bondi and Meyer (4) found approximately 10 per cent methoxyl groups in the lignin isolated from several species of grass. Their work also indicates that each structural unit of the lignin molecule from grasses contains two methoxyl groups which would give a theoretical methoxyl content of 9.9 per cent. Phillips *et al.* (34) found the methoxyl content of the lignin of oats increased from around 4 per cent to approximately 15 per cent during growth to 84 days of age. Similar changes occurred in the lignin of barley plants (33) and timothy plants (32) during development. The percentages of the total methoxyl groups that were associated with the lignin in the four stages of orchard grass hay increasing in maturity were 31.9, 35.1, 40.7, and 45.4 per cent, respectively. Adams and Castagne (1) found 82.4 per cent and 81.7 per cent of the total methoxyl groups of wheat and oat straw, respectively, in the lignin fraction. Phillips and Goss (29) found from 52.0 to 81.1 per cent of the total methoxyl groups associated with the lignin fraction of barley plants at various stages of development. From 39.3 to 85.7 per cent of the total methoxyl content of the oat plant was found in the isolated lignin by Phillips *et al.* (30).

There is not much change, however, in the percentage of the total methoxyl groups which are associated with the lignin isolated from the feces of cows fed orchard grass hay cut at four stages of maturity. This would suggest that there is a higher digestibility of the non-lignin methoxyl in the immature forage, and this is evident in a comparison of the digestion coefficients of the various fractions of the methoxyl groups that are given also in Table 5. Digestibility both of the total methoxyl content of the ration and of the methoxyl groups not associated with the lignin decreases with increasing maturity of the plant, but there is practically no change in the digestibility of the methoxyl groups associated with the lignin.

SUMMARY

The apparent digestion coefficients of lignin in rations containing orchard grass hay cut at four stages of maturity ranged from 3.8 to 16.0 per cent.

The lignins isolated from four stages of orchard grass of increasing maturity had nitrogen contents of 4.25, 4.15, 3.53, and 2.41 per cent. The average nitrogen contents of the lignins isolated from the corresponding feces were 5.19, 4.13, 3.62, and 2.91 per cent. Apparently no degradation of the nitrogen associated with the lignin in orchard grass hay occurred during digestion by dairy cows. There may be errors in the determination of lignin that are unaccounted for in the analysis of materials differing widely in other constituents.

The apparent digestion coefficients for the lignin corrected for nitrogen content were somewhat higher (7.5 to 19.8 per cent) than the digestion coefficients for crude lignin.

The benzoic acid excretion in the urine per 100 g. of lignin fed was lower when the most mature hay was fed than when the three less mature hays were

consumed. There was no relation between the digestibility of lignin and the urinary excretion of benzoic acid.

The total methoxyl content of the hay was lower than the total methoxyl content of corresponding feces on a dry matter basis. The methoxyl contents of the lignin isolated from the various hays was higher than the methoxyl contents of the lignin isolated from the corresponding feces.

The per cent of the total methoxyl content of the hay that was associated with the lignin increased with increasing maturity of the orchard grass. Higher proportions of the total methoxyl content of the feces was associated with the lignin in the feces, and only slight changes in this percentage occurred when hays of a wide range of maturity were fed.

Digestibility of the total methoxyl content and the non-lignin methoxyl content of the ration decreased with increasing maturity of the orchard grass hay. Digestion of the lignin methoxyl groups ranged from 20.3 to 31.6 per cent and showed no consistent trend in relation to the stage of maturity of the hay fed.

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A STUDY OF MILK PASTEURIZATION AT HIGH TEMPERATURES [1]

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The common criterion involving pasteurization efficiency is the destruction of bacteria and of the enzyme phosphatase. Especially heat-resistant test organisms have been isolated and used by several investigators (2, 3, 6, 11, 12). Speck (11) and Speck and Lucas (12) reported on the heat resistance of an organism which Speck tentatively identified as *Micrococcus freudenreichii* (MS 66). This organism was found to resist complete destruction by pasteurization at 143° F. for 30 minutes, and it had a Z value similar to that of the theoretical pasteurization curve.

Quantitative phosphatase tests provide a simple test of pasteurization efficiency. The heat inactivation characteristics of the enzyme have been studied by Sanders and Sager (10), Lear and Foster (7), and Hetrick and Tracy (5), employing a phosphatase test described by Sanders and Sager (9). Results of these studies have been treated graphically so as to make it possible to extrapolate to higher temperatures and shorter holding times than those at which observations were made.

The purpose of this study was to find new time and temperature relationships with pasteurization efficiency equivalent to 143° F. for 30 minutes.

PROCEDURE

Milk was heated in the Mallory small-tube heat exchanger, which was described previously by Herreid *et al.* (4). In these studies a new homogenizer with a capacity of 204 gal. per hour was used as the pump, resulting in a holding time of 2.36 seconds. Only one heating section was used in order to obtain a shorter heat-up time. Temperatures were determined with an iron vs. constantan thermocouple mounted inside the holding tube and a potentiometer sensitive to 0.3° F.

The test organism, *M. freudenreichii* (MS 66), was grown on slants of special medium, as recommended by Speck (11). Transfers were made weekly, and the cultures were incubated at 98° F. Between transfers the cultures were held at about 45° F. To prepare an inoculum, a transfer was made from a slant held between 1 and 6 days into a tube of nutrient broth (Difco) which was subsequently incubated for 24 hours at 98° F. The broth culture was added to the milk at 80° F. and mechanically agitated for 5 to 10 minutes to disperse the organisms. Standard methods were followed for plating, i.e., the use of tryptone glucose extract agar and 48 hours incubation at 32° F.

Survivor curves of the test organism were determined at 140.2° F. and at

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144.4° F. Inoculated sterile milk, 5 ml. per tube, was heated in cork-stoppered test tubes, 18 mm. in diameter, in a thermostatically controlled water bath. The milk was heated to the holding temperature in approximately 1 minute by immersion in water of a slightly higher temperature.

The heating and cooling characteristics of the heat exchanger were determined with water by inserting a thermocouple at each return bend. It required about 0.5 second for the water to pass from one return bend to the next.

Milk inoculated with the test organisms was subjected to various heat treatments in the heat exchanger, and bacterial destruction was measured by means of standard plate counts. Control counts for pasteurization at 143.0° F. for 30 minutes were obtained from samples heated in cork-stoppered test tubes in the manner already described. The plate counts for unheated milk were obtained on milks after passage through the heat exchanger with no heat applied. It was found that this treatment caused an increase in count, probably due to breaking up of clumps. Milk so treated was used also for laboratory pasteurization.

The effect of various heat treatments on the inactivation of phosphatase was determined with the test of Sanders and Sager (9), and the color was measured in a Coleman spectrophotometer.

The standard error of the mean ($s_{\bar{x}}$) for the interpolated equivalent temperatures in the bacteriological and phosphatase data was calculated by means of the equation

$$s_{\bar{x}} = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

where d = deviation from the mean ($\bar{x}$) and n = number of determinations. The confidence limits for $\bar{x}$ are given by

$$\bar{x} \pm ts_{\bar{x}}$$

with t estimated at n – 1 degrees of freedom at the 1% level.

RESULTS

Table 1 gives the changes in temperature with time of water heated to a temperature of 170.4° F. and cooled to 86.7° F.

TABLE 1

The rate of heating and cooling of water in the Mallory small tube heat exchanger

Heating Section		Cooling Section	
Time of flow	Temp.	Time of flow	Temp.
(sec.)	*(° F.)*	*(sec.)*	*(° F.)*
0.0	80.0	0.0	170.4
2.0	88.0	0.5	142.7
2.5	90.2	1.0	124.6
3.0	90.0	1.5	114.3
3.5	103.0	2.0	96.7
4.0	170.4	2.5	86.7
6.36 [a]	170.4		

[a] A 2.36 second holding tube connected the heating section to the cooling section.

TABLE 2

Effect of various temperatures on the destruction of Micrococcus freudenreichii (MS 66) in milk processed in the Mallory small tube heat exchanger

Sample	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Temp. (° F.)	Count (per ml.)	Temp. (° F.)	Count (per ml.)	Temp. (° F.)	Count (per ml.)	Temp. (° F.)	Count (per ml.)	Temp. (° F.)	Count (per ml.)
Uninoculated	80	9,500	80	13,700	80	9,700	80	239,000	80	12,000
Inoculated	80	153,000	80	346,000	80	305,000	80	611,000	80	314,000
Inoculated	163.0	169,000	167.6	71,000	166.0	148,000	166.0	180,000	166.0	119,000
Inoculated	166.0	118,000	169.3	19,600	167.6	81,000	167.6	132,000	168.0	28,000
Inoculated	171.0	8,800	172.7	4,100	169.7	9,800	169.7	35,000	170.0	3,300
Inoculated					172.0	370	170.7	22,000	171.1	880
Uninoculated	143.0 [a]	310	143.0 [a]		143.0 [a]	160	143.0 [a]	6,000	143.0 [a]	380
Inoculated	143.0 [a]	19,900	143.0 [a]	42,000	143.0 [a]	65,000	143.0 [a]	70,000	143.0 [a]	16,800
Interpolated temp. giving equivalent destruction to 143° F. for 30 min.	168.8	(19,900)	168.3	(42,000)	167.8	(65,000)	168.4	(70,000)	168.4	(16,800)

$\bar{x}$ (°F.) = 168.34 $s_{\bar{x}}$ (°F.) = 0.16 Confidence limits at 1% level (° F.) = 167.60 — 169.08

[a] Laboratory pasteurized at 143° F. for 30 min.

TABLE 3

Effect of various temperatures on the inactivation of phosphatase in milk processed in the Mallory small tube heat exchanger

Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
Heating temp.	Phenol	Heating temp.	Phenol	Heating temp.	Phenol	Heating temp.	Phenol	Heating temp.	Phenol
(°F.)	(γ per ml.)	(°F.)	(γ per ml.)	(°F.)	(γ per ml.)	(°F.)	(γ per ml.)	(°F.)	(γ per ml.)
168.3	7.92	169.0	6.05	169.0	7.20	169.0	7.80	167.7	21.60
169.3	4.80	171.0	1.68	169.7	3.00	169.7	4.32	169.9	3.84
170.3	1.44	171.5	1.01	170.0	2.69	170.5	2.35	170.7	0.72
171.7	0.36			170.5	2.40	170.7	1.99	171.7	0.24
				171.7	0.48			172.4	0.00
169.5 [a]	(4.00)	169.8 [a]	(4.00)	169.6 [a]	(4.00)	169.8 [a]	(4.00)	169.8 [a]	(4.00)

$\bar{x}$ (°F.) = 169.70 $s_{\bar{x}}$ (°F.) = 0.063 Confidence limits at 1% level (°F.) = 169.41 — 169.99

[a] Interpolated temperature necessary to reduce the enzyme to 4γ per ml.

Survivor curves of *M. freudenreichii* (MS 66) at 140.2° F. and 144.4° F. are given in Figure 1. Each point on the curves was obtained from counts on the combined contents of three separately heated tubes and thus represent the mean counts of triplicate determinations.

Data on the destruction of the test organisms and the enzyme phosphatase at various temperatures with a holding time of 2.36 seconds are given in Tables 2 and 3, respectively.

DISCUSSION

According to Rahn (8), the thermal destruction of bacteria is closely approximated by the monomolecular law $N = N_0 e^{-bt}$ at constant temperature T where N is the number of organisms at time t and N_0 the number originally present. A plot of $\log \frac{N}{N_0}$ versus time would thus yield a straight line. When $\log \frac{N}{N_0}$ is kept constant, a straight line would result from a plot of log t versus T. The data of Table 2 show the variation of N with T at constant t and a straight line should be obtained from a plot of $\log \log \frac{N}{N_0}$ vs. T. Deviations from linearity have been observed, possibly due to clumping of the organisms at high values of $\frac{N}{N_0}$, and deviation from the first order reaction at low values of $\frac{N}{N_0}$. In the interpretation of the data, a straight line was drawn between the points representing a $\log \log \frac{N}{N_0}$ just smaller and just larger than that for the laboratory pasteurized sample, and the equivalent temperature was obtained by interpolation. This resulted in values which were in good agreement with those obtained when the points were connected by a curve.

The data of Speck (11) indicate that the Z value of *M. freudenreichii* (MS 66) is 8.4° F. A value of 8.2° F. is obtained from consideration of Figure 1 ($Z = \frac{\Delta T}{\log Q_{\Delta T}}$). Since the Z value of the theoretical pasteurization curve is 8.4° F., the equivalent temperature found in this study should agree rather closely with the temperature found by extrapolating the pasteurization curve to 2.36 seconds holding time. The extrapolated value is 167.7° F., as compared to 168.34° F. found in this study.

Recently Speck and Lucas (12) suggested the following equation for calculating various time and temperature relationships for pasteurization:

$$\log t_s = 21.103 - 0.137T + 0.014S$$

where t_s = time in minutes to give 99.99 per cent kill of the test organism, T = temperature in degrees F., and S = per cent solids-not-fat added to the milk above its natural content. For t = 2.36 seconds the equivalent temperature is calculated to be 164.3° F., a value somewhat lower than would be predicted from the Z value of the organism.

The data of Table 3 have been treated in a similar manner. The log log phenol concentration per milliliter was plotted against temperature and the temperature corresponding to log log 4 γ of phenol was obtained by interpolation from the plot. Four γ of phenol per milliliter is Sanders and Sager's (9) recommended criterion for pasteurization. A higher temperature was required to inactivate phosphatase than was found to give bacterial destruction equivalent to laboratory pasteurization at 143° F. for 30 minutes. This is due to the fact that 143° F. for 30 minutes is not sufficient to reduce the phosphatase activity to

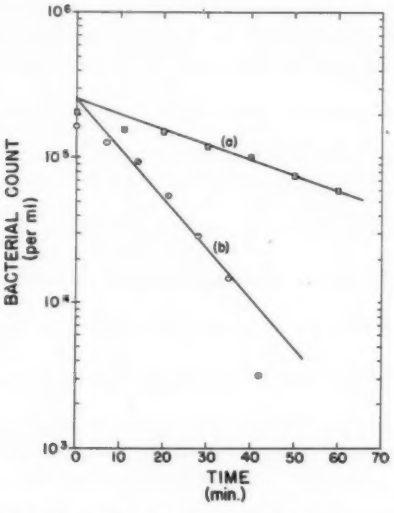

FIG. 1. Effect of time of heating on the destruction of *Micrococcus freudenreichii* (MS66). (a) at 140.2° F., (b) at 144.4° F.

4 γ of phenol and that this enzyme has a slightly higher Z value than the pasteurization curve.

Sanders and Sager (10) found that the following time and temperature relationships yielded 4 γ of phenol: 143° F. for 37.5 minutes, 143.7° F. for 30 minutes, 160° F. for 24 seconds, and 161.8° F. for 15 seconds. A plot of log time versus temperature of these data yields a straight line which may be extended toward higher temperatures. Thus a holding time of 2.36 seconds would require a temperature of 168.85° F. to give a negative phosphatase test. The data of Hetrick and Tracy (5) would predict a temperature of 170.6° F. to reduce the phenol content to 1 γ per milliliter with the same holding time. Results reported in Table 3 indicate a temperature of 169.7° F., which is only slightly higher than the extrapolated value from the data of Sanders and Sager. The difference may be due to the extremely short heat-up and cooling time used in these experiments and to variations in the phosphatase content of raw milk.

In an effort to evaluate the relative safety factor of the equivalent temperatures found in the Mallory heat exchanger, the data of North and Park as interpreted by Dahlberg (1) for *Mycobacterium tuberculosis* were used to calculate lethal effects (reciprocal of thermal death time). In terms of this organism, 161° F. for 15 seconds, which is legal pasteurization, yielded a total lethal effect of 1.65, assuming instantaneous heating and cooling. A temperature of 168.34° F. for 2.36 seconds, the equivalent for *M. freudenreichii* (MS 66), has a total lethal effect of 1.27, whereas 169.7° F. for 2.36 seconds, conditions found necessary to give a negative phosphatase test, has a total lethal effect of 1.65. This method of interpretation has the advantage in that the safety factor in pasteurization may be evaluated. The data of North and Park on *Mycobacterium tuberculosis* point out the smaller safety margin for pasteurization of milk at 161° F. for 15 seconds as compared to 143° F. for 30 minutes. The lethal effects are 1.65 and 4.5, respectively.

CONCLUSIONS

A temperature of 168.34° F. with a holding time of 2.36 seconds in the Mallory small tube heat exchanger was found to give destruction of *M. freudenreichii* (MS 66) equivalent to laboratory pasteurization at 143° F. for 30 minutes. A temperature of 169.7° F. was necessary to reduce the activity of phosphatase to 4 γ of phenol per milliliter.

In terms of the data of North and Park, as interpreted by Dahlberg (1), on the thermal destruction of *Mycobacterium tuberculosis*, 169.7° F. for 2.36 seconds yields the same total lethal effect as 161° F. for 15 seconds, assuming zero heat-up and cooling time in both cases.

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CHANGES IN THE PLASMA LEVEL OF PROTEIN-BOUND IODINE IN THE YOUNG CALF [1]

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A previous report (2) has shown the plasma protein-bound iodine (PBI) level of young calves to be higher than it is in older animals. The reasons for this phenomenon have been investigated and are the subject of this report.

METHODS AND MATERIALS

The analytical procedures have been reported elsewhere (1). The cows and some of the calves studied were from the Michigan State College Holstein and Jersey herds. Other calves were born into the herd of the Ohio Agricultural Experiment Station at Wooster, and samples of their plasma were supplied to this laboratory by Dr. L. O. Gilmore.

RESULTS AND DISCUSSION

Plasma PBI changes in young calves: Table 1 shows the changes in the plasma PBI concentration of calves during the first 18 months postpartum. The average plasma PBI level of 22 calves less than 24 hours old was 14.8 γ per cent. During

TABLE 1
Age changes in the plasma protein-bound iodine concentration of calves

Age	No. of calves	No. of determinations	Average γ %	s
Under 24 hr.	22	22	14.8	5.3
24 - 48 hr.	8	8	10.8	2.3
3 - 4 d.	8	9	7.9	4.2
5 - 7 d.	9	9	7.6	4.1
8 d. - 1 mo.	24	34	6.9	3.6
1 - 3 mo.	18	18	6.2	2.6
4 - 6 mo.	11	17	8.1	3.4
7 - 12 mo.	21	27	7.3	1.6
13 - 18 mo.	10	27	6.8	2.7

the second 24 hours after birth the concentration fell to an average of 10.8 γ per cent. By the third day postpartum the plasma PBI level was only slightly higher than the average of animals up to 18 months of age. At the end of the first week the concentration had decreased to a level comparable to that of the older heifers.

Since the PBI concentration was particularly high during the first 48 hours, it was felt worth while to make a special study of that period. Accordingly, seven

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calves were sampled immediately after birth before they were permitted to nurse and again 6 to 12 hours after birth. As shown in Table 2, a marked rise occurred in the plasma PBI level, which may have been associated with the ingestion of colostrum. At birth these calves had an average plasma PBI concentration of 8.9 γ per cent, but after nursing the level rose to 15.0 γ per cent, a statistically significant increase of over 66 per cent. Although a considerable amount of variation between calves was apparent, these differences may be attributed to differences in the amount of colostrum intake and to variations in the iodine content of the colostrum.

TABLE 2

Plasma protein-bound iodine levels in calves before and after first nursing

Calf No.	PBI Level		Increase
	Before nursing	After nursing	
	(γ %)	(γ %)	(%)
A	7.1	8.2	15.5
B	11.1	12.5	12.6
C	5.9	13.9	101.7
D	9.3	14.9	60.7
E	14.1	29.7	110.6
F	6.9	15.6	126.1
G	7.7	10.5	36.4
Av.	8.9	15.0	66.2

Iodine in colostrum and milk. As there is little information on the iodine content of colostrum and milk, several attempts were made to analyze samples of each for both total and protein-bound iodine. Considerable difficulty was experienced in working with colostrum because of the variability in its organic matter content and the amount of iodine found. The results of several analyses are shown in Table 3. It is evident that colostrum contains a much larger PBI

TABLE 3

The iodine concentration of milk and colostrum

Sample [a] No.	Colostrum [b]		Milk [c]	
	PBI	Total Iodine	PBI	Total Iodine
	(γ %)	(γ %)	(γ %)	(γ %)
1	....	20.2	4.5	12.2
2	....	28.8	6.1	13.6
3	25.0	30.9	....	8.0
4	21.9	....	....	8.1
5	[d]	[d]	5.4	7.2
6	[d]	[d]	....	9.0
7	[d]	[d]	....	9.9
8			7.2	9.3
9			6.1	....
10				6.7

[a] Milk and colostrum samples are not necessarily from the same cows.
[b] Colostrum samples were taken within 6 hr. postpartum.
[c] Milk samples were taken from cows at various stages of lactation.
[d] Iodine concentrations above 35.0 γ per cent.

fraction than does normal milk, although the size of this fraction is quite variable from cow to cow. It was observed that higher iodine values were associated with thick, viscous colostrum and that the PBI fraction rapidly fell to the level in milk.

The nature of the PBI fraction in the initial colostrum is unknown. The normal mammary gland has been shown to be impervious to thyroxine (3). There is no evidence known to the authors which would indicate the existence of a different situation at calving time. There have been no reports of thyroxine accumulation in colostrum.

Since a large protein-bound iodine fraction does exist in the first colostrum, it is interesting to speculate upon its origin. In this connection it is perhaps significant that the plasma PBI level of the dam is lower on the day of calving than it is several days before or after calving (Table 4).

TABLE 4

Changes in plasma protein-bound iodine levels of cows at calving time

Cow No.	Breed	PBI Level		
		Before calving	At calving	After calving
		(γ %)	*(γ %)*	*(γ %)*
5	Jer.	3.8	2.6	4.1
15	Hol.	4.1	1.4	5.0
17	Hol.	3.8	2.5	3.7
101	Jer.	4.5	2.0	4.8
115	Jer.	4.1	3.0	6.7
128	Hol.	4.1	3.5	3.7
132	Jer.	6.3	2.3	5.4
134	Hol.	5.1	2.9	3.9
Av.		4.5	2.5[a]	4.6

[a] Highly significantly lower than levels before or after calving ($T = 5.30$).

The organic iodine in plasma is thought to be associated with the circulating globulins and albumins (5). The first colostrum is much richer in globulin and albumin-like material than is milk, and these proteins appear to be similar to those in the circulation (4). These observations suggest that the PBI in colostrum may be related to its globulin and albumin content. Several possibilities present themselves: *(a)* Plasma proteins and their associated iodine may pass from the circulation into the colostrum in an unchanged form; *(b)* inorganic iodine from the circulation or resulting from the metabolism of the circulating PBI is filtered into the colostrum where it is attached to the proteins; and *(c)* the iodine may represent residual material remaining in the udder as an aftermath of the dry period. Of the three suggestions the second and third appear to be most tenable. The first would be more appealing if colostrum possessed calorigenic properties. Either the first or second suggestion might explain the fall in the dam's PBI concentration at calving time. The third possibility can be supported only by the results of one analysis of the fluid obtained by milking a dry cow. No PBI could be detected, although the total iodine value was high.

Thyroid histology. The histological appearance of the thyroid glands of four calves slaughtered at various intervals after birth is shown in plate 1. A pro-

gressive differentiation of the secretory cells and accumulation of colloid in the follicles which parallels the disappearance of iodine from the circulation may be seen.

These data show that colostrum supplies the new-born calf with relatively

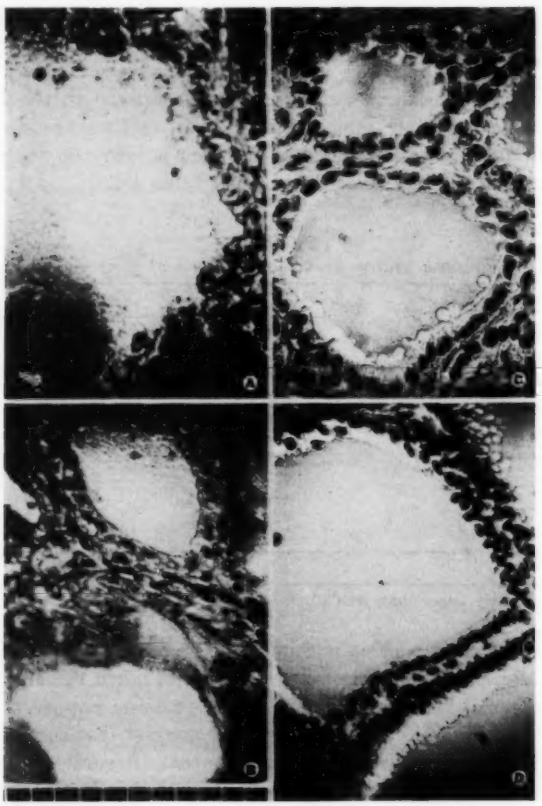

FIG. 1. Photomicrographs of thyroid gland secretions of young calves.

A. The thyroid gland of a 12-hr. Jersey bull. There were few follicles and they were nearly devoid of colloid. Secretory cells were columnar and poorly defined. The calf had a plasma PBI concentration of 23.8 γ percent at thyroidectomy.

B. A 3-day-old Jersey bull. The amount of colloid in the follicles had increased and the secretory cells were more cuboidal than in the younger calf. The plasma PBI concentration at thyroidectomy was 3.1 γ percent.

C. A 4-day-old Jersey bull. Numerous follicles well filled with colloid and continued tissue differentiation may be observed. The plasma PBI concentration was 8.3 γ percent at thyroidectomy.

D. A thyroid section from a 6-day-old Guernsey bull. Follicles were well filled with deep staining colloid. The secretory cells were well defined and no undifferentiated tissue remained in the gland. This calf had a plasma PBI concentration of 3.7 γ percent at thyroidectomy.

All glands presented a normal gross appearance.
Magnification: Each scale division equals 10 microns.

large amounts of protein-bound iodine, which rapidly disappears from the circulation and appears to be stored in the thyroid gland. The importance of this iodine to the calf can only be speculated upon. Since the period immediately after birth must be one of considerable stress for the calf, this may be one of the mechanisms whereby the calf is prepared to meet its new environment.

SUMMARY

The plasma protein-bound iodine concentration of young calves has been found to be much higher during the first 48 hours postpartum than at any later time. This high level appears to be due to the ingestion of colostrum, which was found to contain relatively large amounts of protein-bound iodine during the first 12 hours after calving. Histological evidence is presented showing that large amounts of colloid are stored in the thyroid follicles as the plasma PBI level falls.

It was found also that the plasma PBI concentration of the dam is depressed on the day of calving. The possible significance of this is discussed.

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OBSERVATIONS ON THE CHEMICAL COMPOSITION OF WHITE PARTICLES IN SEVERAL LOTS OF CHEDDAR CHEESE [1]

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The white particles in Cheddar cheese have been reported by Dorn and Dahlberg (2) to consist primarily of tyrosine, whereas Tuckey *et al.* (8) and McDowall and McDowell (5) have found that they are composed principally of calcium lactate. Shock *et al.* (6) showed that the crystalline white particles found in one lot of aged Cheddar cheese were heterogeneous in composition and were composed primarily of a mixture of calcium lactate and tyrosine. Cystine was found to be a third major component. The divergence in the results of different workers indicated the importance of studying white particles from different lots of cheese. Therefore this study was carried out to determine whether or not the white particles from different lots of Cheddar cheese have the same chemical composition.

EXPERIMENTAL

White particles were collected from six different samples of Cheddar cheese. Some particles were collected from the surface of the cheese sample and others

TABLE 1
Description of cheese from which the white particles were obtained

No.	Age in months	Raw or pasteurized milk	Method of ripening	Location of white particles in sample
1	18	Pasteurized	Daisy, ripened at 40° F.	Interior
2	42	Raw	Daisy, ripened at cold temperature	Interior
3	20	Pasteurized	Vacuum packed at 12 months (3″ x 5″ x 1″) cured at 40° F.[a]	Surface
4	18	Pasteurized	Daisy, shelf cured	Interior
5	103	Raw	Vacuum canned in vented glass jars, 1939	Surface
6	150[b]	Raw	Vacuum canned in vented glass jars, 1936	Surface
7	150[b]	Raw	Vacuum canned in vented glass jars, 1936	Interior

[a] Vacuum packed in laminated cellophane type wrapper.
[b] White particles from the same lot of cheese used by Shock *et al.* (6). These were collected from a previously unopened container, 30 months after the samples taken by Shock *et al.* (6).

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from the interior. Table 1 gives a description of the cheese from which these particles were collected and the location of the particles in the sample. A fine dissecting needle was used to pick the white particles from the cheese, using great care to exclude any adhering cheese. White particles from the interior of the cheese were selected only when they were found to be free in the cracks and crevices of the samples. The particles then were washed with ethyl ether to remove any fat. The washed white particles were dried under a vacuum, residual pressure, 10 mm. in a desiccator at room temperature until they attained a constant weight. They were stored in weighing bottles in a desiccator until they were analyzed. Only a few hundred milligrams of material could be collected from most samples.

The particles were analyzed for tyrosine, cystine, and leucine by microbiological assay methods. The methods were those used by Harper and Swanson (3). The particles were analyzed also by X-ray diffraction. The powder diffraction method was the same as that used by Shock *et al.* (6).

Qualitative tests for amino groups (ninhydrin), peptide linkage (biuret), tyrosine (Millons), tryptophane (Adamkiewicz), lactic acid (Strauss), and sulfur were carried out as described by Hawk, Oser, and Summerson (4). The results are shown in Table 2.

All white particle samples showed the presence of compounds containing free carboxyl and alpha amino nitrogen groups. The biuret test indicated that peptides were not present in any of the samples. Tyrosine was found in all samples, but a negative test was obtained for tryptophane. Lactic acid was found

TABLE 2

Qualitative analyses of white particles from a number of samples of Cheddar cheese

Sample No.	Qualitative test					
	Amino groups (Ninhydrin)	Peptide linkage (Biuret)	Tyrosine (Millons)	Tryptophane (Adamkiewicz)	Lactic acid (Strauss)	Sulfur
1	Pos	Neg	Pos	Neg	Pos ?	Pos
2	Pos	Neg	Pos	Neg	Pos	Neg
3	Pos	Neg	?	Neg	Neg	Neg
4	Pos	Neg	Pos	Neg	Pos	Neg
5	Pos	Neg	Pos	Neg	Pos	Pos
6	Pos	Neg	Pos	Neg	Pos	Pos
7	Pos	Neg	Pos	Neg	Pos	Pos

TABLE 3

The quantity of tyrosine, cystine, and leucine in the white particles from a number of samples of Cheddar cheese (all values expressed on dry weight basis)

Sample No.	Quantity of amino acids as per cent of dry weight		
	Tyrosine	Cystine	Leucine
1	49.6	6.1	1.3
2	18.3	None	None
3	3.2	None	8.6
4	11.0	None	0.3
5	31.7	8.6	0.8
6	35.9	13.3	1.1
7	36.1	11.8	1.4

present by the Strauss test in all but one sample, and four of the samples gave a positive test for sulfur.

Table 3 shows considerable variation in the quantitative concentration of tyrosine, cystine, and leucine in the various white particles. This indicates that the particles from different lots of cheese may be expected to vary in chemical composition. No relationship was found between the amino acid composition and the use of raw or pasteurized milk. The method of ripening does appear to have some influence on the quantities of tyrosine, crystine, and leucine found in the white particles. Because of the very small samples available, complete compositional analyses were not undertaken.

The "d" spacings found upon X-ray diffraction of standards and white particles are shown in Table 4. Standards were run for C. P. calcium lactate and purified tyrosine and cystine.

The standards were washed with ether and dried in the same manner as that used for the white particles. The "d" spacings as shown in Table 4 vary considerably between samples. A relationship between the number of identifying spacings for a given compound and the concentration of the compound in the

TABLE 4

X-ray diffraction patterns of known standards and white particle samples

"d" spacings in Å for each of the following:									
Calcium lactate	Tyrosine	Cystine	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
11.88			11.88	11.90	14.54	11.92	11.88	11.90	11.87
9.80			9.79	9.80		9.83	9.80	9.83	9.80
8.06			8.02	8.09		8.01	8.05	8.00	8.06
	6.55		6.55				6.55	6.54	6.57
6.01			6.01	6.01		6.03	6.00	6.01	6.01
	5.84		5.80				5.81	5.84	5.84
	4.90		4.90	4.91			4.89	4.90	4.91
4.86				4.86		4.84		4.84	4.86
		4.70	4.74						4.70
4.40			4.40		4.57			4.41	4.40
	4.09		4.10			4.07	4.06	4.09	4.08
4.03			4.05	4.03					4.02
		4.00						4.01	3.98
	3.60		3.60		3.64	3.62	3.59	3.61	3.60
3.58									
3.26				3.26			3.24	3.27	
		3.21	3.22						
		3.14	3.10	3.08				3.14	3.15
3.08							3.04	3.08	3.09
	2.93		2.93	2.94		2.92	2.90	2.94	2.94
2.82		2.74	2.75	2.84			2.82	2.82	
		2.60			2.62	2.62	2.74	2.78	2.78
	2.45		2.45	2.45		2.44	2.47	2.43	2.45
2.40			2.40	2.41			2.40	2.39	2.40
		2.35				2.38			2.35
		2.15					2.15	2.15	
	2.03		2.02					2.04	2.03
		2.01						1.82	
	1.83		1.83					1.76	1.84
		1.78					1.78		1.75
		1.57	1.57						
		1.35						1.36	1.35

sample is apparent; this is particularly true of tyrosine. All of the major "d" spacings for tyrosine are found in sample 1, which contains nearly 50 per cent tyrosine; only one-third of the characteristic spacings are found in sample 2, which contains 11 percent tyrosine; and none of the characteristic spacings are found in sample 3, which contains only 3.2 percent tyrosine. Smith and Barrett (7) have reported that one of the major limitations of X-ray diffraction analyses is that about 10 percent of the compound being investigated must be present in the sample. In some cases this value may vary from 1 per cent to 50 per cent before positive identification is possible. The results in Table 4 substantiate the findings of Smith and Barrett and demonstrate that X-ray diffraction should be supplemented by chemical analyses. When characteristic "d" spacings are present in the pattern in sufficient numbers, positive identification is possible, but compounds present in concentrations of less than 10 per cent may be overlooked.

The data in Table 4 demonstrate the presence of tyrosine and calcium lactate in all samples except No. 3, the number of spacings for tyrosine being roughly proportional to the tyrosine concentration. Cystine is demonstrated in samples 1, 5, 6, and 7. The spacings for sample 3 do not correspond with either tyrosine, cystine, or calcium lactate. Three of the four spacings correspond closely to those reported for pure isoleucine by Tuckey *et al.* (9), and the remaining spacing of 14.54A might be compared to leucine at 14.2A. Microbiological assay for leucine showed about 8 per cent in this sample, which was considerably higher than that found in any other sample. Lack of sufficient sample prevented further microbiological analyses. Leucine had been reported in white particles of cheese during the 1800's by several workers (1, 10). Since isoleucine was not discovered until 1903, the early reports of leucine particles may have involved isoleucine. The fact that sample 3 had been packaged in a laminated wrapper under vacuum may have been a factor in producing white particles of a composition quite different from those found in the other samples.

SUMMARY

The data in Tables 2, 3, and 4 reveal that the white particles selected from a number of different samples of Cheddar cheese are definitely heterogeneous in composition. The compounds found most commonly were tyrosine and calcium lactate. Quantitative analyses for tyrosine show that its concentration varied from 3 per cent to 50 per cent in the samples studied. Cystine was found in the white particles from three of the six samples of cheese. The white particles from one cheese sample contained only a small amount of tyrosine and yielded no X-ray diffraction evidence of calcium lactate. This sample did contain appreciable quantities of leucine, and isoleucine was probably present.

The study included four raw milk and three pasteurized milk cheeses; there is no indication that this factor influenced the composition of the white particles. No significance could be attached to the location of the white particles in the cheese in regard to their composition. The method of ripening appears to have a definite influence on the composition of the white particles.

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CALCIUM METABOLISM IN DAIRY COWS AS STUDIED WITH Ca^{45}

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Investigations on the mechanism of Ca mobilization and its secretion into milk are receiving new impetus from the availability of radiocalcium for research purposes. The use of this radioisotope makes possible an approach to various lactational problems in the intact animal without resort to nonphysiological concentration levels. Methods of analysis for radiocalcium in biological material have been developed by Comar *et al.* (3), and procedures for the use of radioisotopes with large animals have been described by Hansard (10) and Hansard *et al.* (11). Basic information on the behavior of Ca^{45} in beef cattle has been reported by Hansard *et al.* (12). Visek *et al.* (20) have reviewed the literature on Ca^{45} studies in lactating ruminants and have carried out such investigations with lactating goats. No reports have been found on the use of Ca^{45} in research with the dairy cow, probably because of the lack of suitable techniques and the high cost of experimental animals, which are not usually salvageable after studies of this type. The high cost of animals has been minimized in the studies here presented by the repeated use of the same three cows at various stages of the lactation cycle. This procedure has the additional advantage of comparing the response of an individual in various physiological states.

Data are presented as to the excretion of Ca^{45} and its secretion in milk after oral and intravenous administration to the cow, both as a single dose and in multiple daily doses. Information is included also on the existence of various Ca fractions in the blood and on the behavior of Ca^{45} administered shortly before parturition, which gives information on the appearance of this element in colostrum.

The use of radioisotopes has raised problems of interpretation which have not been encountered heretofore in conventional biochemical studies. Therefore, there are emphasized certain aspects of interpretation which must be taken into consideration if labeled element methods are to yield the information of which they are inherently capable.

EXPERIMENTAL METHODS

With the exception of variation in feeds, the procedures were similar for all phases of the work. Grade cows served as experimental subjects; cow 1 was a 4-year old Guernsey and cows 4 and 5 were 8-year old Jerseys. Feeding was carried out according to Morrison's recommendations (15). Intravenous injection was done by jugular catheter and oral administration by gelatin capsule, as described by Hansard (10) and Hansard *et al.* (11). For intravenous injection

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the solution of $Ca^{45}Cl_2$, which had been maintained at high acidity previous to dosing to prevent the precipitation of Ca as the hydroxide, was adjusted to pH 6 immediately prior to injection.

With the exception of prepartum and continuous oral dose studies, balance studies were made. Complete feed consumption records were kept, and refusals were sampled for analyses. In every case the animals underwent preliminary conditioning in metabolism stalls, during which time they were submitted to the dietary regimen which was to be followed during the course of the experiment. Fecal and urine samples were taken daily, and sampling intervals for blood and milk were dictated by the particular experimental procedure employed. In all experimental samples, both total and radioactive Ca values were determined from the oxalate precipitate, as described by Comar *et al.* (3). Feed analyses were carried out according to A.O.A.C. methods (1). Complete milking was accomplished by injection of 10 I.U. of oxytocin prior to dosing and whenever milking intervals were less than 12 hours.

All results were calculated in terms of percentage of the dose administered. Hence, the term "specific activity" as used in this paper is expressed as the per cent of the Ca^{45} dose times 10^4 per milligram of total Ca; the factor of 10^4 was used for convenience of expression. The per cent of the dose in the total blood at any one time was calculated by assuming that the blood comprised 7.7 per cent of the body weight (5).

It should be emphasized at the outset that the departure from usual isotope methodology employed here — the use of one animal for repeated administration of radioactivity — was carefully controlled. In every case samples were analyzed before dosing to make sure that the residual radioactivity from previous experiments was negligible.

RESULTS

Studies at Parturition: Cows 1 and 4, in their dry periods, received Ca^{45} intravenously at 20 and 22 days before parturition, respectively. Cow 1 received 15 millicuries of activity in 0.42 g. of total Ca, and cow 4 received 5 millicuries in 0.65 g. of Ca. The animals were bled at intervals after injection in order to follow the activity picture in the blood. After parturition, accurate weights of colostrum and milk were taken. The calves were prevented from nursing at birth and each calf was bled before it was allowed to consume any colostrum. These steps were taken to obtain a comparison of the activity in the blood of the dam with that of the newborn calf.

Figure 1 shows the specific activity of the blood and milk plotted as a function of time after administration for blood and after freshening in the case of colostrum and milk. Since the results for both cows were very similar, only the data for cow 1 are presented. In both animals the specific activities of the milk were decidedly higher than those of the blood at the time of parturition. This differential tended to persist until about the 7th day after freshening in cow 4 and about the 15th day in cow 1. These results indicate that much of the radiocalcium found in colostrum probably entered the mammary gland at a

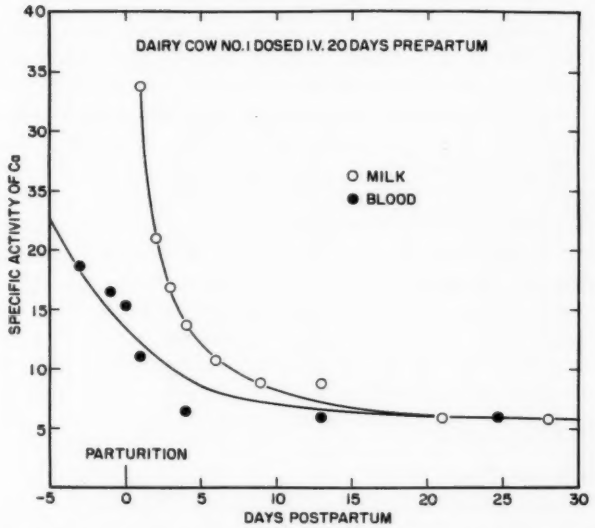

FIG. 1. The specific activities of the calcium in blood and milk plotted as a function of time after intravenous injection of Ca^{45} at 20 days prepartum.

time when the specific activity of the blood Ca was high and remained there until the animal was milked. Just what percentage of the Ca secreted in the colostrum and milk was being obtained from the skeletal stores and what percentage came from possible stores in the mammary gland cannot be determined from these results.

In these investigations it was noted also that the specific activities of the Ca in the serum of the calves at parturition were two and one-half times as high as those of their dams. This indicates that the Ca^{45} crossed the placental barrier from dam to fetus and did not, thereafter, come into equilibrium with the Ca in the maternal circulation.

Intravenous studies during lactation: Cow 1 was injected with Ca^{45} three times during lactation and cow 5, once. Table 1 presents information on the ration, treatment of animals, and the results. Excretions were sampled daily; blood and milk samples were taken as indicated.

Table 1 summarizes the percentage of injected Ca^{45} appearing in the milk and feces. In all studies the peak secretion into the milk of injected Ca^{45} occurred between 8 and 24 hours after administration. Cow 1 secreted about 32 per cent of the dose in 4 days in approximately 30 kg. of milk which she produced in that time; when her milk production dropped to 17 kg., the per cent of Ca^{45} dose secreted dropped to about 19 per cent. However, the values for Ca^{45} per kilogram of milk and the Ca^{45} per gram of milk Ca did not show these proportional variations and were considered constant within the experimental error. Cow 5, following her 280th day of lactation, produced 25 kg. of milk in 4 days

TABLE 1

Appearance of Ca^{45} in the milk and feces of dairy cows after single intravenous injection

	Cow No.			
	1	1	1	5
Body wt. *(lb.)*	690	722	708	796
Postpartum *(d.)*	86	147	305	280
Ca^{45} injected *(millicuries)*	5.0	14.9	1.75	1.75
Ca injected *(g.)*	0.21	0.5	0.04	0.04
Ca^{45} excreted in feces in 4 d. *(%)*	16.7	18.0	15.6	20.5
Milk produced in 4 d. *(kg.)*	30.1	29.3	16.6	25.4
Milk produced in 7 d. *(kg.)*	59.1	53.3	.....	.....
Ca^{45} in milk *(% dose accumulated)*				
2 hr.	3.7	4.6		
8 hr.	9.8	11.5		
1 d.	20.9	23.5	9.1	20.3
2 d.	26.5	29.7	14.1	28.8
3 d.	30.1	31.4	17.0	33.0
4 d.	32.5	32.3	18.7	35.7
7 d.	35.8	33.7		
Ca^{45} per kg. of milk over 4 d.period *(% of dose)*	1.08	1.10	1.13	1.40
Ca^{45} per g. of Ca in milk over 4 d. period *(% of dose)*	1.06	0.88	0.83	0.94

Cow 1 was fed concentrate mixture containing 18% crude protein plus U. S. No. 2 Orchard Grass hay in the first two trials; during the third trial she was fed mixture I and Red Clover hay, to be described later. Cow 5 received the same ration as Cow 1 in the third trial plus 300g. of steamed bone meal daily which was fed to prepare her for another study. Cow 1 at 305 days postpartum and Cow 5 received Ca^{45} on the same day.

and secreted 36 per cent of the Ca^{45} dose in her milk. The milk of cow 5 actually had a higher Ca content than that of cow 1, and when the values were calculated on the basis of Ca^{45} deposition per gram of total Ca, there was excellent agreement between the two animals.

It would appear that these two cows, at least, secreted injected Ca^{45} in direct proportion to the total amount of Ca being secreted into the milk. Except for the variation of Ca concentration in milk from different animals, the percentage of the injected Ca^{45} secreted by any cow would depend on her level of milk production. This behavior is in contrast to that of the kidney and intestine, which eliminate relatively constant amounts of Ca regardless of the total amount of feces or urine excreted, as shown in these and other studies (12). The bonemeal supplement fed to cow 5 apparently had no effect on the results obtained. No correlation with either production level or total Ca secretion was observed by Visek *et al.* (20) in their studies with goats, but the fact that they injected rather large amounts of calcium may have added a complicating factor.

Fecal excretions, as noted in Table 1, expressed in per cent of the dose, ranged from 15.6 to 20.5 for the 4-day period. Less than 2 per cent of the dose was excreted in the feces after the 4th day. Most of the fecal excretion occurred within 24 hours after injection. Four-day urinary excretion values expressed as per cent of the dose for cow 1 were 0.46, 1.20, and 1.45 at 86, 147, and 305 days postpartum, respectively, and 1.04 for cow 5. The amount and pattern of fecal excretion was similar to that observed by Hansard *et al.* (12) for beef cattle, although the urinary values here reported were somewhat higher.

Figure 2 presents the specific activities of Ca in blood and milk plotted as a function of time after dosing for cow 1 at 147 days in lactation. This curve is typical of all four trials. As observed by others (12, 19, 20), the activity of blood declined rapidly after injection. About 18 per cent of the injected Ca^{45} remained in the total blood after 30 minutes, 8.5 per cent after 2 hours, and approximately 1 per cent after 24 hours. Of particular interest are the relationships between the specific activities of Ca in blood and milk. The specific activity curve for the milk increased rapidly, crossed the declining blood specific activity curve in 1 to 1½ hours after dosing, reached a maximum about 2 hours later, then declined exponentially at a slightly faster rate than the blood specific activity and finally reached equilibrium with the blood at about 100 hours. With minor variations these curves resembled the precursor-product curves of Zilversmit *et al.* (22). The interpretation of these data will be considered later.

Single Oral Administration: It seemed worthwhile also to study the metabolism of orally administered Ca^{45} from the standpoint of estimating the absorption from the tract, and also to obtain more information on the possible mechanism of transfer of Ca from blood to milk under conditions where the blood level of Ca^{45} was not changing so rapidly. Cow 5 was given orally 3 millicuries of Ca^{45} in 1.12 g. of total calcium, and her milk and blood were sampled at 4-hour intervals over the critical time range. Blood samples were drawn from a venous catheter to prevent excessive trauma to the animal.

Presented in Figure 3 are the specific activities of blood and milk plotted as a function of time after administration. It is evident that the blood specific activity reached a maximum at about 30 hours after administration, and the maximum for milk was attained at approximately 37 hours. The time differences

TABLE 2

Rations fed to dairy cows receiving Ca^{45} orally

	Mixture I	Mixture II
Ingredients of grain mixture	*(lb.)*	*(lb.)*
Yellow corn, ground	49.9	20
Oats, ground	25.0	25
Wheat bran	19.0	19
Soybean oil meal (s.p.)	5.0	35
Salt	0.8	1
Steamed bone meal	0.3	0
Total	100	100

Composition of grain mixtures and clover hay fed

	Mixture I	Mixture II	Clover hay
	(%)	*(%)*	*(%)*
Dry matter	89.1	89.8	93.6
Crude protein	12.3	24.5	11.6
Ether extract	3.2	2.7	1.8
Crude fiber	6.0	6.6	30.8
Ash	5.9	4.0	4.9
Nitrogen-free extract	61.7	52.0	44.5
Calcium	0.22	0.23	0.19
Phosphorus	0.62	0.78	0.38

required to reach these maxima may be due in part to a time lag in the conversion of blood Ca to milk Ca. The fact that the milk attained a specific activity higher than was ever encountered in the serum is difficult to explain. One implication of these results, of course, is the possibility that a Ca-containing blood fraction of relatively high specific activity and slow turnover rate was being preferentially utilized in the milk secretion process. Consequently, blood fractionation studies were undertaken with this view in mind.

These studies on oral absorption of Ca^{45} provided an opportunity to determine whether there was an influence of the level of dietary protein on Ca absorption, as suggested by McCance *et al.* (14) for man. Accordingly, a single reversal experiment was set up, using cows 1 and 5 with adequate and high levels of dietary protein in the grain mixture. The composition of these mixtures is presented in Table 2, and dosage and treatment schedules are presented in Table 3.

TABLE 3

Dosage and treatment of animals receiving single oral dose of Ca^{45}

Cow	Postpartum d.	Body Wt.	Dose fed		Protein in grain mixture
			Ca^{45}	Ca.	
		(lb.)	*(millicuries)*	*(g.)*	*(%)*
1	228	694	4	0.57	12.3 (I)
5	203	906	4	0.57	24.5 (II)
1	276	684	5	0.82	24.5 (II)
5	251	898	5	0.82	12.3 (I)

TABLE 4

Partition of Ca^{45} following oral administration to lactating cows fed on normal and high levels of dietary protein

Time after feeding	Percentage of Ca^{45} dose			
	Cow 1		Cow 5	
	Normal protein	High protein	Normal protein	High protein
Ca^{45} in total blood				
1 d.	0.42	0.62	0.59	0.37
2 d.	0.37	0.38	.....	0.48
3 d.	0.26	0.26	0.39	0.37
4 d.	0.22	0.20	0.23	0.19
5 d.	0.18	.....	.....	0.15
6 d.	0.09	0.09	0.16	.....
7 d.	0.04	.....	.....	0.06
Ca^{45} in daily milk				
1 d.	0.70	0.83	0.58	0.07
2 d.	1.53	1.59	1.48	2.00
3 d.	1.13	1.62	2.39	2.37
4 d	0.83	0.78	1.58	1.88
5 d.	0.56	0.56	1.23	1.36
6 d.	0.44	0.35	0.92	1.00
7 d.	0.25	0.30	0.84	0.69
TOTAL	5.44	6.03	9.02	9.37
Total Milk in 7 d. *(kg.)*	33.1	29.7	39.2	49.2
Ca^{45} in feces — 7 d.	73.0	94.0	71.0	63.0
Ca^{45} in urine — 7 d.	0.35	0.49	0.11	0.34

Table 4 summarizes the distribution of radiocalcium in the blood, milk, feces, and urine for a period of 7 days following each tracer dose. Peak radioactivity in the blood was reached 24 to 48 hours following oral administration; thereafter, the activity declined and reached background levels by the 6th day. The peak appearance of Ca^{45} in the milk occurred during the second or third day after ingestion. The values obtained for fecal and urinary excretion in these studies agreed closely with the values for beef cattle (12), with the exception of cow 1 on the high protein ration, which probably can be ascribed to a condition of temporary diarrhea in this animal. There were no apparent effects from the level of dietary protein.

Blood Calcium Fractionation: For blood Ca fractionation studies the method of Benjamin and Hess (2) was modified to fit our conditions. In essence, the procedure consists of obtaining diffusible and nondiffusible serum Ca by ultrafiltration and further partition of each of these fractions by adsorption on $BaSO_4$.

Samples of jugular blood were drawn and allowed to clot under refrigeration. Separation of the serum was accomplished by centrifugation within 4 hours after the blood was drawn. Samples of whole serum were immediately shaken with $BaSO_4$ for 2 hours, and the ultrafiltrates were subjected to this treatment after 16 to 20 hours filtration through cellulose acetate casings. The time of filtration and use of cellulose membranes were modifications of the Benjamin and Hess method, which were found not to influence our results. The $BaSO_4$ fractions were separated by centrifugation and radioactivity, and chemical analyses were carried out on all fractions. Calculations were made according to the methods of Benjamin and Hess (2), and corrections for volume of serum proteins were made using published values (18). Analyses were made on the fractions from 30 samples of blood from various animals used in these investigations.

The results of these fractionation procedures showed that the Ca^{45} in the sera of the lactating bovine could be divided into four fractions. Table 5 shows

TABLE 5
Calcium Fractions in Cow Sera

			Adsorbable Complex			
	Total	"Protein bound"	Total	Filtrable	Non-Filtrable	Remainder (ionized)
Specific Activity	25.4	24.1	26.2	26.3	25.6	28.8
Ca *(mg/100 ml. serum)*	11.3	5.5	5.0	4.1	0.9	0.8

that the specific activities of all fractions were the same within limits of experimental error. The values are averages of 12 determinations in which the counting rates for all fractions were sufficiently high for reliable measurement. *In vitro* experiments showed that within a few minutes after Ca^{45} was added to eight serum samples the specific activity of the filtrable Ca fraction was within 4 per cent of the specific activity value of the bound Ca fraction. Further fractionation by adsorption on $BaSO_4$ was thought to be unrewarding for this experiment and consequently was not carried out. Other workers also have observed this rapid and uniform distribution of Ca^{45} in serum (8). Thus there seems little likelihood

of the existence of a high specific activity fraction, and even if in *in vivo* conditions did allow the maintenance of such a fraction, ionic exchange would probably cause equilibrium conditions before the fractional separation could be completed. Therefore it is not possible to preclude entirely the existence of a high specific activity fraction in the blood.

This rapid attainment of equilibrium between the calcium of the various fractions isolated may be utilized to provide a simple way of estimating the proportional amount of serum Ca in each fraction. This is normally done by determining chemically the total Ca in each fraction, which requires a relatively large amount of each fraction and is time consuming. The same results can now be obtained easily by determination of the total serum Ca, addition of some Ca^{45}, separation of the fractions and simple measurement of the Ca^{45} in each fraction.

Daily oral administration during lactation: In the studies previously discussed Ca^{45} was administered to dairy cows in single oral or intravenous doses. For comparison, a study was conducted employing daily ingestion over several months of a lactation period. Also, it was thought that such an experiment would yield information concerning the eventual equilibrium between the specific activities of blood and milk. This type of experiment with Ca^{45} has not been found previously reported in the literature.

Cow 4, an 8-year old grade Jersey cow weighing 824 lb., began receiving Ca^{45} orally by gelatin capsule on the 64th day of lactation in daily doses of 150 microcuries in 3 mg. of Ca. A fair quality mixed hay of orchard grass and Johnson grass and a commercial concentrate mixture containing 18 per cent total crude protein were fed. Ca and P intakes were adequate according to recommended allowances, and the animal was maintained in an isolated paddock of about one acre where adequate shelter was provided. Samples were taken of the morning and evening milkings. During the first 10 days, milk and jugular blood samples were taken daily and thereafter on the same day each week. Body weights were obtained monthly and were found to vary not more than 5 per cent.

Figure 4 presents the specific activities of the Ca in the blood and milk plotted as a function of time after the initial dosing. The results showed considerable fluctuations in specific activities of blood and milk, but both tended to vary in the same directions together. The ratio of the specific activities approached unity after the first 40 days of the experiment. This supports the view that the specific activity of the blood precursor of milk Ca was the same as that of the total blood, since Zilversmit *et al.* (22) have pointed out that after coninuous administration the ratio of the specific activities of a product and its precursor(s) ultimately reach unity. On the other hand, the values over the last 150 days of the experiment indicate that the specific activity of the milk was maintained at a slightly higher level than that of the blood, thus leaving open the question of a high specific activity fraction in blood.

DISCUSSION

The role of blood constituents as precursors for milk constituents has been of interest for many years. Unlike fats, sugars, and proteins, some of which are

unique to milk and are synthesized in the lactating cell, Ca undergoes chemical change only insofar as it is associated with various biochemical compounds. It would be expected that the time course of the specific activities of Ca in blood and milk would show the precursor-product relationship as described by Zilversmit *et al.* (22). However, deviations from this relationship were noted in Figure 2, where the curves crossed before the milk reached its maximum specific activity,

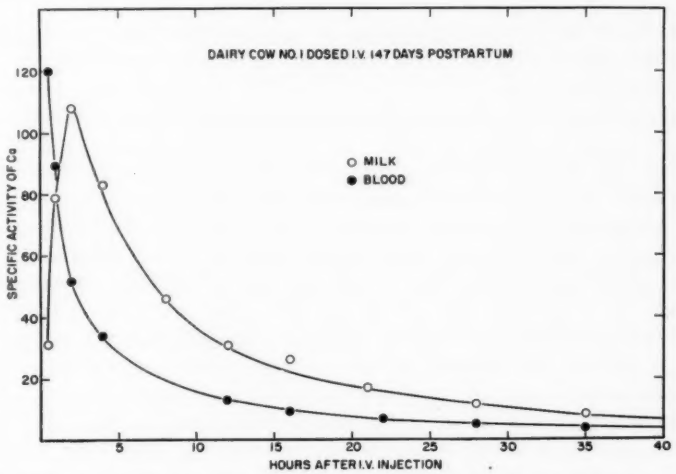

FIG. 2. The specific activities of the calcium in blood and milk plotted as a function of time after intravenous injection of Ca^{45}. Ca^{45} administered 147 days postpartum.

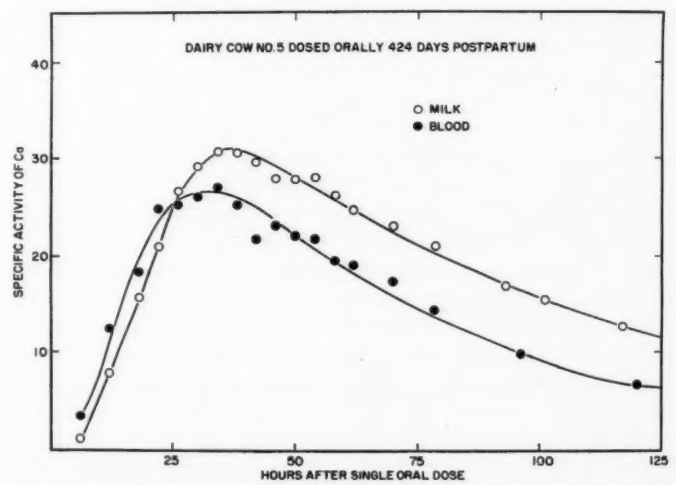

FIG. 3. The specific activities of the calcium in blood and milk plotted as a function of time after oral administration of Ca^{45}.

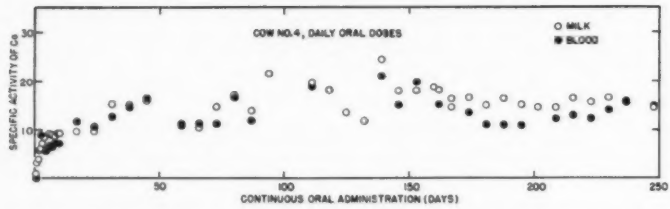

FIG. 4. The specific activities of the calcium in blood and milk plotted as a function of time following daily feeding of Ca^{45}.

and again in Figure 3, where the milk attained a specific activity higher than that ever encountered in the serum.

Several possible explanations for this behavior have been considered. A careful study of the possible errors in sampling and analyses has eliminated these as a source of discrepancy. Consideration next was given to the possibility that the relationship observed was accounted for by a simple time lag in the transport of Ca from blood to milk. Theoretically the specific activity in a milk sample should be equal to the average specific activity of the blood from which it was derived. Analysis of the data, however, has indicated that in many cases the specific activity in the milk sample was equal to that in the blood taken at a time several milkings previously. This seems to rule out the simple time lag of milk formation, even if one considers the difficulties in sampling the milk which is representative of blood at any one time. The residual milk remaining in the udder following milking after oxytocin injection would not be sufficient to account for the time differential observed, although it might contribute.

Another possible explanation is based on the existence of a mammary metabolic pool of Ca, as suggested by Gowen and Tobey (9), who found that the ash content of mammary gland was higher than could be accounted for by the milk present. If radio-calcium from blood entered such a pool and then was randomly used in production of milk, the time lag could be accounted for, depending on the size and turnover rate of the pool. Milk proteins and various colloidal complexes probably are concerned in maintaining this pool (17, 21).

We should like to postulate as a possible explanation the well-recognized removal of radiocalcium from blood by the exchange process with bone (4, 7, 13, 19). This exchange process could make it possible for the blood in the mammary gland at any given time to be of higher specific activity than jugular blood at the same time. There is experimental evidence to indicate that blood leaving bone has less Ca^{45} and, therefore, a lower specific activity than that which entered it (4). Before orally administered Ca reaches the jugular vein, it first must reach the heart via the portal vessels and posterior vena cava and then, after passage through the lungs, be pumped through the general systemic circulation. During this process, some of the Ca must have passed through the vessels of bone prior to reaching the sampling point in the jugular vein. Simultaneously, Ca of specific activity equal to that reaching the bone will reach the mammary gland where some of it is removed by the milk secretion process. This would account not only

for the time lag observed but also for the high specific activities found in the milk as compared with blood. This same behavior was observed by Saarinen *et al.* (16) for the transfer of phosphate from blood to milk. Their results also might be explained by this exchange phenomenon, which has been shown to occur with phosphate as well as with Ca (6).

SUMMARY

Radiocalcium was used to study Ca metabolism in dairy cows at parturition and at various stages of lactation. Data are presented on the appearance of Ca^{45} in the blood, milk, feces, and urine at various times following single oral and intravenous doses. The blood-milk pattern also is given for a cow fed Ca^{45} daily over several months of a lactation period.

When Ca^{45} was injected into two dairy cows in single doses at about 20 days prepartum, there was an indication that much of it was deposited in the mammary glands, where it remained until the animals were milked. Radiocalcium intravenously administered during lactation was deposited in the milk in amounts directly correlated with the total amount of milk calcium secreted. During 4 days after injection, a cow producing about 4 kg. milk per day secreted 19 per cent of the Ca^{45} dose, whereas the same cow and another one producing about 7 kg. per day secreted 34 per cent. Following oral administration these cows secreted from 4 to 6 per cent of the Ca^{45} in their milk in 4 days at daily levels of milk production which were about the same as those following intravenous injection.

Evidence is presented that Ca ions are readily interchangeable between the various Ca fractions of blood, and on this basis a simple method for quantitative estimation of these fractions is suggested. These data on Ca fractions, as well as the blood and milk specific activity relationships following oral and intravenous administration, are presented and discussed in terms of precursor-product relationships.

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SUGAR AND ACIDS IN GRASS SILAGE[1]

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What becomes of the sugar, either naturally occurring or added, when forage crops are ensiled? In the past it has been rather generally assumed, with little or no experimental evidence to fortify the assumption, that in the normal fermentation process in silage the sugar is all, or substantially all, converted into short-chain fatty acids and carbon dioxide. Some work has been done at this station in the past year to shed more light on the problem.

EXPERIMENTAL

Thirty-seven lots of forage crops were ensiled in June, 1951. Five of these were stored in a 125-ton concrete silo; the other thirty-two were put up in steel drums of approximately 250-lb. capacity, sealed at the top with plastic covers held in place by giant elastic bands cut from old inner tubes. The five lots stored in the large silo were separated by markers, and samples from them were identified further by the "buried bag" technique.

Two different types of crop were represented. In the large silo and in 16 of the drums the crop was a mixture of grasses (timothy, bluegrass, and brome grass) and legumes (red clover and alsike clover), in which the grasses predominated. The legumes were in bloom when ensiled; the grasses were headed but not yet in bloom. In the other 16 drums the crop was a nearly pure stand of red clover (in bloom) plus a little alfalfa, orchard grass, and quack grass. Moisture content at ensiling time was 75.7 per cent and 82.1 per cent, respectively. The two types will be referred to as grass silage and legume silage, respectively.

The large silo was opened in November, the first sample from it being taken on December 6, and the last one on March 31. The small containers were opened one at a time over a period extending from November 20 to April 23. Preservatives or conditioners used are listed in Tables 1 and 2. Approximate rates of application per ton of green crop were: molasses, 90 lb.; hominy meal, 150 lb.; citrus meal, 150 lb.; ground oats, 140 lb.; dried whey, 100 lb.; dried beet pulp, 150 lb.; sulfur dioxide, 4 lb. Extensive analyses of all samples were made and will form the basis of a later report. Sugar was determined by the Lane-Eynon method as described in *Official Methods* (2). Acids were determined by the methods specified in an earlier paper from this station (1).

RESULTS AND DISCUSSION

Results are summarized in Tables 1 and 2, the former dealing with percentages of the several entities and the latter with actual amounts. Because certain constituents, notably total acidity and lactic acid, were of a markedly different order

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TABLE 1

Total sugar and acids in grass silage [a]

Part 1. From a mixture of grasses and legumes (grasses predominant).

Preservative or conditioner	Number of lots	Percentage of sugar		Percentage of acidity in the silage				
		In the freshly ensiled crop	In the resultant silage	Total	Acetic	Butyric	Lactic	Amino
None	3	3.90 (3.44–4.37)	0.28 (0.27–0.28)	15.67 (13.4–17.8)	6.34 (4.0–7.8)	4.26 (3.9–4.8)	none	5.55 (4.3–6.5)
Molasses	3	12.37	0.68 (0.67–0.69)	14.90 (12.5–18.2)	4.61 (2.8–6.2)	3.31 (1.2–4.5)	3.10 (0–9.3)	3.78 (3.0–5.0)
Hominy meal	3	4.19	0.79 (0.77–0.80)	18.54 (18.2–18.9)	4.64 (4.2–5.0)	0.17 (0–0.26)	7.68 (6.3–10.1)	5.29 (3.7–6.2)
Citrus meal	3	8.47	1.66 (1.33–1.98)	15.51 (14.1–16.8)	4.12 (3.5–5.2)	0.72 (0–1.37)	5.89 (2.4–8.9)	4.10 (3.2–5.1)
Ground oats	2	2.33	0.83 (0.71–0.94)	16.94 (15.9–18.0)	5.98 (5.8–6.1)	0.21 (0.08–0.34)	3.78 (2.1–5.5)	5.27 (5.1–5.5)
Dried whey	2	10.65	2.21	19.54 (17.9–21.2)	3.55 (3.5–3.6)	none	12.38 (10.5–15.2)	4.11 (3.9–4.3)
Dried beet pulp	2	2.88	1.00	16.88 (16.5–17.3)	5.49 (4.6–6.4)	0.09 (0–0.17)	6.25 (4.7–7.8)	4.09 (3.5–4.7)
Sulfur dioxide	3	3.44	4.22 (3.69–4.75)	12.77 (12.1–13.0)	0.58 (0–1.3)	2.70 (1.9–3.4)	5.20 (4.4–5.9)	4.42 (4.3–4.5)

[a] All values are expressed on a dry matter basis.

TABLE 1 (Continued)

Total sugar and acids in grass silage

Part 2. From a nearly pure stand of legumes.

Preservative or conditioner	Number of lots	Percentage of sugar		Percentage of acidity in the silage				
		In the freshly ensiled crop	In the resultant silage	Total	Acetic	Butyric	Lactic	Amino
None	2	6.46 (6.2–6.8)	0.49 (0.44–0.53)	25.58 (23.0–28.2)	4.90 (4.7–5.1)	0.06 (0–0.11)	12.50 (11.3–13.7)	7.59 (6.3–8.9)
Molasses	2	15.75 (15.4–16.1)	2.69	26.56 (25.9–27.2)	5.04 (4.9–5.2)	none	14.82 (13.1–16.6)	6.71 (6.2–7.3)
Hominy meal	2	5.37 (5.3–5.4)	0.47 (0.41–0.52)	23.34 (22.6–24.1)	4.34 (4.1–4.6)	none	12.04 (11.6–12.5)	5.86 (5.5–6.2)
Citrus meal	2	10.56 (10.5–10.6)	2.07	21.74 (20.9–22.6)	4.69 (3.9–5.5)	none	13.18 (11.5–14.9)	4.87 (4.7–5.1)
Ground oats	2	4.88	1.27	27.30 (26.9–27.7)	4.61 (4.5–4.7)	0.03 (0–0.05)	15.39 (14.8–16.0)	7.33 (7.3–7.4)
Dried whey	2	15.83 (14.4–17.3)	3.51	24.24 (23.8–24.7)	3.80 (3.6–4.0)	none	15.57 (15.2–15.9)	5.41 (5.2–5.6)
Dried beet pulp	2	5.53 (5.3–5.8)	1.84	23.83 (21.2–26.5)	5.84 (4.6–7.1)	0.18 (0–0.36)	11.86 (10.8–12.9)	5.41 (5.1–5.7)
Sulfur dioxide	2	6.76	2.46	20.51 (18.8–22.3)	3.87 (3.3–4.4)	0.36 (0.27–0.45)	10.88 (10.0–11.8)	5.24 (4.9–5.5)

The values in parentheses indicate the range: where they are not given it means that only one value is available, the samples having been composited. With such a small number of samples the range is of more significance than the standard error.

Summary

Grasses	21	6.0 ± 1.4	1.5 ± 0.5	16.4 ± 0.7	4.4 ± 0.6	1.4 ± 0.6	5.5 ± 1.3	4.6 ± 0.3
Legumes	16	8.9 ± 1.6	1.9 ± 0.4	24.6 ± 0.8	4.6 ± 0.2	0.08 ± 0.01	13.3 ± 0.6	6.1 ± 0.4
Differences		2.9 ± 2.1	0.4 ± 0.6	8.2 ± 1.1	0.2 ± 0.6	1.3 ± 0.6	7.8 ± 1.4	1.5 ± 0.4

The differences which are statistically significant are those for total acidity, lactic acid, and amino acids, the first two mentioned being highly significant.

TABLE 2

Acid production relative to amounts of sugar in the silage
(expressed as pounds per ton of dry matter)

Preservative or conditioner	Sugar		Residual sugar as a percentage of the original amount	Acids in the silage			Sugar equivalent in the acids formed	Percentage excess of sugar equivalent over the original amount present
	In the freshly ensiled crop	In the resultant silage		Acetic	Butyric	Lactic		
Part 1. From a mixture of grasses and legumes.								
None	78	5.6	7.2	127	85	none	127	62.8
Molasses	247	13.6	5.5	92	66	62	156	—63.2
Hominy meal	84	15.8	18.8	92	3.4	154	217	158.3
Citrus meal	169	33.2	19.6	82	14	118	180	6.5
Ground oats	47	16.6	35.3	120	4.2	76	159	238.3
Dried whey	213	44.2	20.8	71	none	248	296	—34.3
Dried beet pulp	58	20.0	34.5	110	1.8	125	200	244.8
Sulfur dioxide	69	84.4	122.3	12	54	104	138	100.0
Part 2. From a nearly pure stand of legumes.								
None	129	9.8	7.6	98	1.2	250	316	145.0
Molasses	315	53.8	17.1	101	none	296	364	15.6
Hominy meal	107	9.4	8.8	87	none	241	299	179.4
Citrus meal	211	41.4	19.6	94	none	264	327	55.0
Ground oats	98	25.4	25.9	92	0.6	308	370	277.6
Dried whey	317	70.2	22.1	76	none	311	362	14.2
Dried beet pulp	111	36.8	33.2	117	3.6	237	317	185.6
Sulfur dioxide	135	49.2	36.4	77	7.2	218	273	102.2

of magnitude in the two kinds of crop, results are presented in two sections in both tables. Also, since values for total acidity include amino acids derived from protein breakdown and not from sugar, the columns for total acidity and amino acids have been omitted from Table 2. The main points of interest brought out by the tabular summary are:

1. With one exception (legumes with hominy), lots which had preservatives added showed somewhat higher levels of residual sugar in the silage than did the control lots to which no preservative was added. This ranged from about 2.4 times higher (grass with molasses) to 15 times higher (grass with sulfur dioxide), the average being four times higher for the grasses and nearly five times higher for the legumes.

2. The sugar content of the grass-sulfur dioxide lots needs further comment. These were the only lots in which the percentage of sugar was higher in the silage than in the original material. There are two possible explanations. Some of the complex carbohydrates in the crop may have been converted into sugar instead of into organic acids or, what seems more probable, the sulfur dioxide may have protected the sugar originally present from breakdown, so that the apparent increase in sugar is due to loss of other constituents, the amount of sugar remaining undiminished. It is of interest to note that this was not the case with legumes preserved with SO_2; the residual amount of sugar, although relatively high (2.46 per cent) was only slightly over one-third of that contained in the fresh, unensiled crop.

3. The low values for total acidity and the extremely low values for acetic acid in the grass-sulfur dioxide lots are of interest. Most of the other total acidity values in these lots were higher than the controls, some of them substantially so. The lowest acetic acid value in the other grass lots was more than six times the value found when SO_2 was used. Here again the situation did not hold for the legumes, as the value for acetic acid when SO_2 was used, although not as high as most of the others, was of the same general order of magnitude.

4. The unorthodox behavior of the grasses preserved with SO_2 with regard to butyric acid content warrants more than passing comment. Excepting molasses, all preservatives were quite effective in keeping butyric acid at a low level, but when SO_2 was used, the butyric acid content was nearly two-thirds that of the control and over 11 times higher than the average of the others (molasses excepted). Normally, butyric acid does not develop to any appreciable extent unless the silage has a pH around 5.0, in which case lactic acid content usually is relatively low.[2] Contrary to this well-established finding, the average pH of the grass-sulfur dioxide lots was 4.4, and their lactic acid content was over 5 per cent. It seems evident that the type of fermentation which took place in these silages was quite different from that normally occurring in the ensiling process. It has been noted already that the sugar content of these lots of silage was higher than that of the original material. It seems probable that the SO_2 acted to prevent the breakdown of sugar by inhibiting the action of those microorganisms which selectively use sugar as a source of energy, and that organisms which normally attack cellulose or other carbohydrates more complex than sugar were not restricted in their activity by the action of the SO_2. As before, the legume silages did not follow this peculiar pattern, their average butyric acid content being of the "trace" order and not significantly higher than that noted with other preservatives.

5. It is of interest to note the considerable amounts of residual sugar in most of the lots where preservatives were used, the only exceptions being the grass-molasses and the legume-hominy lots. Although molasses furnished a high level of sugar in the material as ensiled, in the grass-molasses lots this disappeared almost entirely during the fermentation process. In the hominy lots the original level was not high, presumably because of the starchy nature of the material. Evidently the sugar-loving microorganisms had so much free scope in the grass-molasses lots that, unlike all the other lots, the sugar originally present accounted for more than all the aliphatic acids present in the silage. In this connection it should be noted that in all lots except grass-molasses, some, and in eight cases most, of the aliphatic acids came from sources other than sugar. Outstanding in this respect were the three low sugar carriers — hominy meal, ground oats, and dried beet pulp — silages from which contained on the average more than three times as much sugar equivalent in the form of aliphatic acids as they originally contained of sugar.

[2] In a recent correlation study of a large number of silage analyses made at this station, butyric acid was shown to be very closely correlated with high pH ($r = .72$) and with low levels of lactic acid ($r = -.64$). Both of these values of "r" are highly significant at the 1% level.

6. The considerable differences in the composition of the grass and legume silages and in the differing reaction of the crops to the several preservatives, and more especially towards SO_2, are perhaps the most outstanding features of this study. The legume silages had a total acidity content 1.5 times higher, a butyric content only one-seventeenth as high, and a lactic acid content 2.4 times higher than the grass silages. The differences in total acidity are statistically highly significant and are doubtless traceable in part to a considerably higher sugar content in the original crop. Such differences indicate silages of quite different character and probably of different feeding value. A similar situation was noted in our earlier work (1).

SUMMARY

Sugar and acid values for 37 lots of silage made in 1951 and sugar values for the original green crops (plus preservatives or conditioners) from which the silages were made are summarized and reported.

Variations in the values for the several lots are discussed, and possible explanations are offered. Of most interest are the apparently quite different types of fermentation that took place in the silage when sulfur dioxide was used as a preservative and the marked difference in amounts of the different organic acids, especially lactic acid, in grass silages as compared with legume silages.

In most cases, sugar equivalent in the silage in the form of the aliphatic acids (acetic, butyric, and lactic) was much above what could be accounted for by the sugar content of the freshly ensiled crop.

Considerable amounts of residual sugar were present in almost all the lots of silage when they were sampled and fed out.

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METHODS FOR STUDYING FACTORS THAT INFLUENCE THE SANITARY CONDITION OF MILKING MACHINE TEAT-CUP LINERS [1, 2]

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Reports on milking machine sanitation frequently indicate that the physical condition of the rubber parts is important in achieving satisfactory results from a bacteriological standpoint. These references apparently pertain to obvious conditions such as freedom from gross dirt, milkstone, cracking, swelling, and softening of the rubber. More recent reports (1, 2, 3) suggest renewed attention to the rubber as a special harbor of bacteria. Jensen and Bortree (7) consider that, under practical conditions, bacteria in teat-cup liners may be lodged in the pores of the rubber. It seems possible that rubber liners, even though in apparently acceptable physical condition, may possess characteristics that are important in sanitation problems. Therefore, consideration should be given to such possibilities in methods used for investigating problems in milking machine sanitation.

For determining the bacteriological conditions of milking machines Ruehle *et al.* (12) devised the "artificial udder" method, which was used subsequently by others (4, 5, 9 ,10). The procedure involves the "milking" of sterile water from the "udder." It presumably achieves a flexing of the rubber teat-cup liners, simulating the action of the machine under practical operating conditions. Bacterial counts of the water are used to represent the amount of contamination from the machine in an equivalent amount of milk during normal milking. A modification of the procedure has been used recently by Wolverton and Cone (13).

Rinse and swab methods also have been employed to measure the bacteriological condition of teat-cup liners. Since no vacuum is applied and there is ordinarily no flexing of the rubber, it might be expected that counts obtained by such methods would differ from those obtained by the "milking" technique. In both the "milking" and rinse procedures it has been shown that repeated treatments remove additional numbers of bacteria from the parts examined.

In addition to methods for estimating the bacteriological condition of milking machines, it is desirable sometimes to contaminate teat-cup liners and other milking machine parts experimentally in the laboratory. Such procedures permit some control in the amount and uniformity of contamination and facilitate laboratory study of certain sanitation problems before final investigation under practical conditions. The artificial "udder" can be used for this purpose by "milking" through milk of high bacterial content. Lineham (8) has devised an apparatus to "milk" controlled quantities of liquid of known bacterial content through individual liners. His device also can be used for measuring bacteriological conditions of individual liners.

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In studies at this Station on a procedure for washing teat-cup assemblies, great variations in bacteriological results frequently were found among individual liners. Accordingly, methods were devised for investigation of factors influencing the sanitary condition of teat-cup liners. In developing the methods, recognition was given to the possible influence of the porosity and microphysical condition of the rubber on bacterial contamination. This report describes the apparatus and procedures and gives results of investigations on their suitability.

APPARATUS

Apparatus for experimental contamination of teat-cup liners. It seems probable that, under practical conditions of operation, distortion and stretching of rubber might be factors in the penetration of bacteria into surfaces of teat-cup liners. In experimental contamination, therefore, it was desirable that the laboratory procedure simulate as closely as possible the normal action of the liners

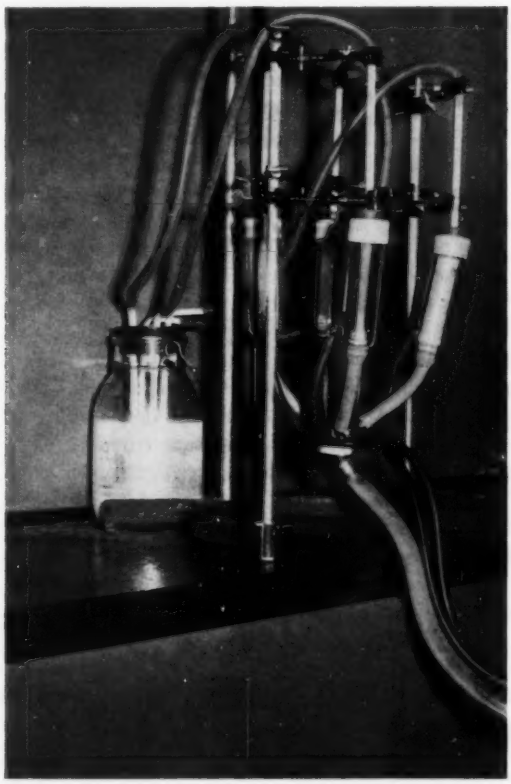

FIG. 1. Artificial "udder" arrangement for experimental contamination of teat-cup liners. Claw connected to portable milking machine.

in usage. Accordingly, the method of "milking" from an artificial udder was utilized. Since some details of the apparatus of Ruehle *et al.* (12) were not reported, and in order to utilize readily available laboratory materials and to permit observation of milk flow into individual teat-cups, the arrangement devised was that shown in Figure 1.

The "teats" consisted of lengths of glass tubing with one end of each tube cemented into the upper half of a rubber stopper. A small orifice about 0.5 mm. in diameter in the lower half of the stopper formed the "teat" opening. The "teats" were held in the desired positions with clamps on a stand. The "udder" was a 1-gal. jar with four pieces of glass tubing extending through the cover to the bottom of the jar. An additional small hole in the cover provided an air inlet. Connections between the "udder" and the "teats" were made by rubber tubing supported as shown.

In operation, the teat-cup assembly was supported on a ring stand and connected to a portable milking machine in the usual manner. The rubber stoppers of the "teats" were fitted into the mouth openings of the teat-cups. The stoppers were of a size (No. 6 or 7) to fit tightly and not be drawn down into the liners by the vacuum of the machine in operation. Three liters of raw milk at about 95° F., previously incubated to have a high bacterial content, were placed in the "udder" jar. By operating the milking machine in the usual manner, milk was drawn through the teat-cup assembly into the milker pail at a rate of slightly less than 1 gal. per minute. The glass tubing permitted observation of the flow of milk into each liner. A transparent plastic milk line provided for additional observation of the milk flow. Glass teat-cup shells, as shown in the figure, were used occasionally to demonstrate the pulsating action of the liners. After laboratory contamination in this manner, the teat-cup assembly was disengaged and removed for study.

Pulsation-vacuum apparatus for determining the bacteriological condition of individual teat-cup liners. It was considered that a method utilizing a flexing action with suction might be more effective than rinse techniques in removing bacteria from liners in counting procedures. Also, such a method simulates the action of liners in practical operation. Therefore, the apparatus devised for obtaining bacterial counts on individual liners involved this feature (Figure 2).

The claw of the teat-cup assembly, connected to a portable milking machine in the usual manner, was supported by a ring stand. The teat-cups were stoppered at the mouth openings with rubber stoppers, inverted, and supported individually as shown. Thirty-five ml. of sterile water was added to each liner. Eight-in. lengths of glass tubing were connected to the stem ends of the inverted liners. Connections to the milk inlet nipples on the claw were made with 24-in. lengths of rubber tubing, which were supported about 20 in. above the stoppered mouths of the liners. The teat-cup shells were connected directly to the air inlet nipples of the claw in the usual manner. The rubber stoppers, rubber tubing, and glass tubing were sterilized in a steam cabinet before each use.

With the machine running, the reduced pressure and pulsating action caused the water in each liner to surge up and down in the liner and the glass tubing.

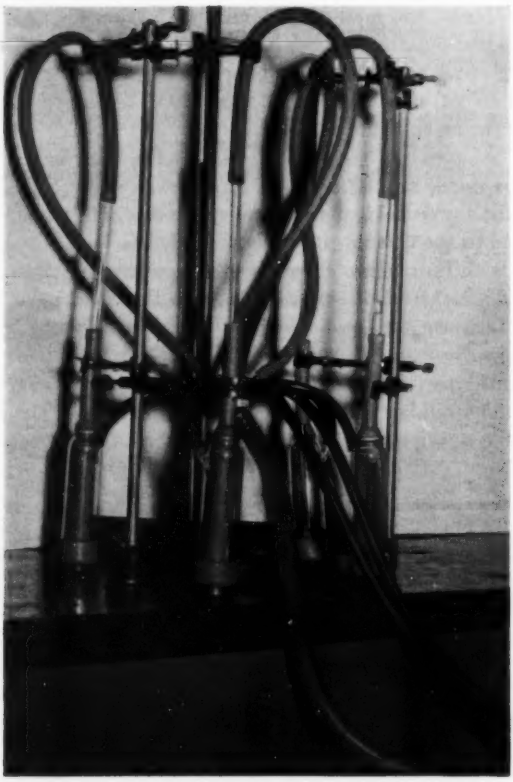

FIG. 2. Arrangement for bacteriological examination of individual teat-cup liners. Claw connected to portable milking machine provides pulsation and vacuum.

The water was prevented from going over into the claw and milk tube by the elevation of the rubber connecting tubing. With a 20-in. elevation, the 35-ml. quantity of sterile water was found to be about the maximum amount that could be used without some being drawn over into the machine pail. Glass teat-cup shells, shown in Figure 2, frequently were used to demonstrate the pulsating action of the liners. After the desired period of surging action (5 minutes, unless otherwise stated), the machine was stopped and the teat-cups disconnected. Plate counts were made on the water in each liner, using Standard Methods and tryptone glucose extract agar.

Apparatus for determining the bacteriological condition of the teat-cup assembly. For obtaining bacterial counts on teat-cup assemblies, the artificial "udder" was used with 3 l. of sterile water "milked" from sterilized "udder" parts through the liners, claw, and milk tube. The arrangement was the same as shown in Figure 1, but sterile water was used in place of incubated raw milk.

Instead of drawing the water into the milker pail, it was by-passed into a trap jar which could be sterilized more conveniently than the pail, pail head, and parts. Water from the trap jar then was plated in the usual manner. To obtain a bacterial count on the liners only, as a unit, the milk tube and claw also were steam sterilized.

EXPERIMENTAL PROCEDURES

Tests on suitability of the experimental contamination technique. In order to determine whether experimental contamination of liners would give the same general magnitude of contamination as that which occurs under practical operating conditions, bacteriological examinations were made on liners contaminated under both conditions. The liners to be contaminated experimentally first were cleaned and steam sterilized. After contamination, they were removed from the claw and rinsed briefly under a cold water faucet to remove free milk droplets. The liners were drained and examined individually, using the pulsation-vacuum technique previously described. The naturally contaminated liners were obtained from the College dairy barn shortly after milking and before they were washed. In the laboratory, the teat-cup assembly was flush-washed in mild detergent solution at about 110° F. to remove obvious dirt and free milk. The assembly was dismantled, and the liners were examined bacteriologically in the same manner as the experimentally contaminated liners.

Since a large part of subsequent investigations involved individual liners, it was desirable to know the extent of variation that might occur in bacterial counts of four different liners experimentally contaminated at the same time. For this purpose liners that were new, or in similar physical condition as determined microscopically, were employed. After cleaning and steam sterilization, the liners were contaminated and then examined bacteriologically, using the pulsation-vacuum method.

In order to determine the uniformity of contamination obtained between sets of four new liners contaminated from the same lot of milk, bacteriological examinations were made by the teat-cup assembly unit method. Only the liners were considered, and the claw and milk tube were sterilized.

Tests on efficiency of the pulsation-vacuum technique for bacteriological examination of liners. Trials were conducted to determine whether the pulsation-vacuum method for making bacteriological examinations on individual liners was more effective than a rinse-shake procedure. In making the rinse-shake count, the mouth opening of the liner was closed with a sterile stopper. The liner was inverted and 35 ml. of sterile water was added through the stem end. The end of the stem was bent backward to close the opening and the liner was shaken vigorously 25 times through a distance of 10 to 12 in. Portions of the rinse then were removed for plating. After the rinse count, the liners, still containing the same rinse, were arranged and connected as shown in Figure 2 for examination, using the pulsation-vacuum procedure. After 1-minute and 5-minute periods of operation, portions of the water were removed for plating.

The effectiveness of the pulsation-vacuum procedure in removing bacteria

from liners was tested further in trials involving three separate successive counts on liners following a single experimental contamination treatment. In each count, the period of pulsation-vacuum action was 3 minutes.

Some exploratory trials with the pulsation-vacuum procedure were made, using a wetting agent in the sterile water rinse to determine whether increased bacterial counts would be obtained. Tween 40 (0.005 per cent to 0.05 per cent in water) and Tween 80 (0.025 per cent in water) were used in comparison with water having no added wetting agent. In each trial, eight previously sterilized liners were contaminated experimentally in the same manner from the same lot of high count milk. The liners were new or in similar physical condition as determined microscopically. In each trial individual counts were made on four liners with sterile wetting agent solutions and on the other four with sterile water only.

RESULTS

Bacterial counts on teat-cup liners contaminated experimentally compared with those contaminated under practical conditions. The majority of counts on the experimentally contaminated liners and the naturally contaminated liners fell in the same general range (Table 1). The experimental group, however, had

TABLE 1

Bacterial counts on teat-cup liners contaminated experimentally compared with those contaminated under practical operating conditions

Bacterial count per ml. of water rinse [a]	No. of liners examined	
	Experimentally contaminated	Naturally contaminated
0-24	11	3
25-99	27	15
100-499	15	25
500-4,999	25	22
5,000-9,999	6	6
10,000-19,999	0	4
20,000-200,000	4	1
Totals	88	76

[a] Bacteriological examinations made by the pulsation-vacuum procedure for individual liners; 35 ml. water rinse used per liner.

a slightly higher proportion of liners with counts in the extremes of the range. Since no special effort had been made to use milk of the same bacterial content for contaminating the liners in different trials, such counts are not unexpected. In addition, the generally satisfactory physical condition of the naturally contaminated liners, together with the flush-washing treatment, tended to minimize the number of unusually high counts in these liners. The bacteriological condition of liners contaminated by the method of "milking" from an artificial udder could be maintained in a range sufficiently similar to that of naturally contaminated liners for experimental work.

Variations in bacterial counts within sets of four liners experimentally contaminated at the same time. Since different lots of incubated milk were used

TABLE 2

Variations in bacterial counts within sets of four liners experimentally contaminated at the same time.[a]

Series	Trial	Bacterial counts on liners per ml. of water rinse[b]			
		1st liner	2nd liner	3rd liner	4th liner
Low contamination	1	30	40	25	40
	2	5	7	10	5
	3	800	800	250	300
	4	55	35	20	70
	5	300	200	550	920
	6	50	32	73	23
	7	20	25	15	12
	8	20	15	15	50
High contamination	9	5,400	6,000	3,800	8,400
	10	2,300	6,400	3,800	3,400
	11	27,000	86,000	120,000	97,000
	12	3,400	7,800	9,500	9,200
	13	81,000	70,000	45,000	75,000

[a] Contaminated by the artificial "milking" method. The different trials did not necessarily include the same liners.

[b] Bacteriological examinations made by the pulsation-vacuum procedure for individual liners; 35 ml. water rinse used per liner.

in the different trials, the amount of contamination in the liners covered a wide range (Table 2). Within each trial, however, the counts on the individual liners were reasonably consistent. Although in one trial the highest count was about four times as great as the lowest count, in others it was only about twice as great. For the 13 trials the highest count averaged less than three times as great as the lowest count. Although the differences may seem large in some cases, the variations are not great when the characteristics of bacterial counts are considered. The counts on individual liners were sufficiently uniform to indicate that experimental contamination would be useful in studying factors affecting liner sanitation.

TABLE 3

Variations in bacterial counts between sets of four liners experimentally contaminated from the same lot of milk.[a]

Trial	Bacterial counts on sets of 4 liners per ml. water "milked" through unit.[b]	
	1st Set	2nd Set
1	975	550
2	125	230
3	325	750
4	125	185
5	400	420
6	850	520
7	38	60

[a] Contaminated by the artificial "milking" method. The 2nd set was contaminated immediately following the 1st set. The different trials did not necessarily include the same set of liners.

[b] Bacteriological examinations made by the "milking" procedure for teat-cup assemblies. Three liters of sterile water used. Only the liners were considered; the other parts of the assembly were sterilized.

Variations in bacterial counts between sets of four liners experimentally contaminated from the same lot of milk. Results of seven trials are presented in Table 3. In each trial, the counts on the two sets were approximately similar and showed even less variation than was found previously between individual liners. In all but one trial the highest count was less than twice as great as the lowest count. The bacterial counts on sets of four liners contaminated from the same lot of milk were sufficiently similar to facilitate investigations involving comparative studies.

Comparison of bacterial counts made on liners by a rinse-shake procedure and by the pulsation-vacuum procedure. Results of comparisons on 56 experimentally contaminated liners are summarized in Table 4. The action of the pulsation-

TABLE 4

Comparison of bacterial counts made on liners by a rinse-shake procedure and by the pulsation-vacuum procedure.

(Liners experimentally contaminated)

Trial	No. of liners	Log av. count per ml. of water rinse per liner [a]		
		Rinse-shake procedure [b]	Pulsation-vacuum procedure [c]	
			1 min.	5 min.
1	8	28	109	272
2	8	2,178	.....	24,200
3	8	164	2,265	5,850
4	8	154	931	949
5	8	125	708	1,219
6	8	36	.....	162
7	8	10	18	87

[a] Each liner examined individually using 35 ml. water rinse.

[b] Stoppered liners containing water rinse were vigorously shaken 25 times through distance of 10 to 12". Rinse portions were then removed for plating.

[c] Following the rinse-shake procedure, the liners were connected as described under "Methods" and pulsated. Water rinse portions were removed for plating after 1 minute and 5 minute intervals.

vacuum procedure resulted in higher bacterial counts than did the rinse-shake method. When the liners were subjected to the pulsation-vacuum action for one minute following the rinse procedure, the bacterial counts increased considerably. The increases in the log average counts for the eight liners in each trial ranged from 80 per cent to more than 1,200 per cent, with a tendency for the percentage increases to be greater with the higher counts. The average increase was more than 700 per cent. An additional 4 minutes of the pulsation-vacuum action gave a further increase. At the end of the 5-minute treatment, the increases over the rinse count ranged from more than 300 per cent to 3,500 per cent, with an average increase of 1,100 per cent.

Successive bacterial counts obtained on the same liners using the pulsation-vacuum procedure. Of the total numbers of bacteria obtained by three successive counts on liners after a single experimental contamination treatment, the first, second, and third count represented about 50, 30, and 20 per cent, respectively (Table 5). Presumably, additional numbers of bacteria still remained in the liners. Although the pulsation-vacuum procedure was more effective than the

TABLE 5

Successive bacterial counts obtained on the same liners using the pulsation-vacuum procedure.[a]

(Liners experimentally contaminated)

Trial	No. of liners	Log av. count per ml. of water rinse per liner.[b]		
		1st count [c]	2nd count [c]	3rd count [c]
1	8	350	200	150
2	8	243	111	75
3	8	21,000	12,460	8,855
4	8	200	150	40

[a] Successive counts followed a single contamination treatment.
[b] Each liner examined individually using 35 ml. water rinse.
[c] In each count the pulsation period was 3 minutes with fresh sterile water rinse used each time.

rinse-shake method, it was evident that counts obtained by a single treatment represented only a portion of the total number of bacteria present.

The effect of wetting agent in the sterile water on bacterial counts obtained on liners using the pulsation-vacuum procedure. A summary of the results of 72 counts is presented in Table 6. Both a low count and a high count series were

TABLE 6

Effect of wetting agent in the sterile rinse water on bacterial counts obtained on liners using the pulsation-vacuum procedure.

(Liners experimentally contaminated)

Series	Trial	Count per ml. of water rinse, log av. for 4 liners [a]	
		Sterile water rinse	Sterile water rinse and wetting agent [b]
Low contamination	1	14	50
	2	72	53
	3	119	81
	4	48	43
	5	98	45
	6	17	41
High contamination	7	25,000	18,000
	8	36,000	25,000
	9	3,000	2,000

[a] Each liner examined individually using 35 ml. of water rinse.
[b] Trials 1 and 2, 0.005% Tween 40; trial 3, 0.05% Tween 40; trial 4, 0.025% Tween 40; trials 5 to 9 inclusive, 0.025% Tween 80.

involved. No advantage was gained from the wetting agents used. Except in two trials, the wetting agents seemed to have a slight depressing effect on the counts. However, with the normal range found in individual counts and the possible variations in original contamination, the differences are of doubtful significance. At the lowest concentration of wetting agent used, relatively little foaming occurred during the surging action in the liners. At the highest concentration, considerable foaming resulted, and greater amounts were not tried.

DISCUSSION

In checking the uniformity of contamination achieved by the artificial "milking" technique, it is to be recognized that discrepancies may arise from either the contaminating procedure or the method used for bacteriological examination. These variations could have either a cumulative or a compensating effect. Since there seems to be no way of measuring the extent of contamination without a counting procedure, the exact contamination cannot be known. Even with an apparatus such as Lineham's (8), where equal amounts of inoculum are milked through separate liners, there is no certainty that equal numbers of bacteria will adhere to each liner. Although experimental contamination is a valuable aid for study of factors affecting the sanitary condition of liners, final observation should be made under practical conditions.

The pulsation-vacuum method devised for bacteriological examinations on individual liners has definite merit. It removed considerably more bacteria from the liners than the rinse-shake procedure. The eleven-fold increase obtained over the rinse-shake count was much greater than that reported by Rowlands *et al.* (11) for repeated rinse treatments on milking machine clusters. These workers found that in five successive rinse counts only 50 per cent of the total count was obtained by the last four rinses. In spite of the apparent efficiency of the pulsation-vacuum procedure, however, bacteria still remained in the liners after repeated treatments. Evidently, the organisms adhere tenaciously to the rubber or are imbedded in it.

Although it had been expected that wetting agents would assist in the removal of bacteria from the surface of liners and give increased counts, the results obtained did not show sufficient promise to justify use. It is possible that more extensive studies would have resulted in the selection of a compound and concentration that would give higher bacterial counts from liners. However, the results obtained were in general accordance with the report of Fuller and Sparkes (6), who found wetting agents to be of no particular value in routine plate counting procedures.

The methods devised were subsequently employed in investigating certain characteristics of the rubber surface in relation to teat-cup liner sanitation. The results of these studies will be presented in a later report.

SUMMARY

Since surface characteristics of rubber, combined with physical manipulation, may influence bacterial contamination of teat-cup liners, methods developed for sanitation studies incorporated a flexing action of the liners under partial vacuum, as occurs in practical operation.

A modified "artificial udder" and "milking" technique was utilized for the contamination of liners in the laboratory. Bacterial counts of liners contaminated by this means were in the same general range as that of liners contaminated under practical conditions. Contamination was reasonably uniform in each of the four liners in a set and in separate sets of four liners contaminated from the same lot of milk.

A method was devised for determining the bacteriological condition of individual liners. The partial vacuum and pulsating action of the milking machine were utilized to surge sterile water up and down in the liners. Plate counts then were made on the water. The procedure gave much higher bacterial counts than a rinse-shake method. The use of wetting agents did not increase the counts obtained.

For bacteriological examination of sets of four liners and teat-cup assemblies and milk tubes, modifications of the technique of milking sterile water from an artificial udder and plating the water were used.

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ANTIBIOTICS IN DAIRY CATTLE NUTRITION. II. EFFECTS OF FEEDING AN AUREOMYCIN PRODUCT (AUROFAC 2A) TO LACTATING DAIRY COWS[1, 2]

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The feeding of small amounts of either aureomycin or aureomycin products (Aurofac) to young dairy calves reduced the incidence of scours, increased the growth rate, improved physical condition, and probably improved feed efficiency (3). These benefits apparently continue for some time after the calf has passed from a nonruminating animal to a ruminating one (1, 6). Therefore, since young ruminants benefit from aureomycin feeding, does it follow that supplementing rations of dairy cows with aureomycin will result in increased milk production? To answer this question the following investigation was initiated.

EXPERIMENTAL PROCEDURE

Eighteen cows (ten Holsteins, four Ayrshires, and four Guernseys) were paired as similarly as possible with regard to breed, age, stage of lactation, and production of fat-corrected milk. The cows in each pair then were placed at random in one or the other of two groups. A double reversal design of feeding was used to compare two rations which were similar except for the addition of aureomycin to the grain mixture of one of the groups. The general design of the experiment was as follows: Cows in group I were fed aureomycin during period I, received no aureomycin during period II, and again received aureomycin during period III. Conversely, cows in group II received no aureomycin during period I, received aureomycin during period II, and received no aureomycin during period III. The experimental periods were of 6 weeks' duration. Prior to the start of period I, all cows were maintained under experimental conditions for 8 days. The source of aureomycin was Lederle's Aurofac 2A, a residual fermentation product in the manufacture of pure aureomycin. This product contained 8 mg. of aureomycin per gram and was fed once daily by adding it to the grain mixture, at the rate of 40 g. per 1,000 lb. body weight. This level was selected since preliminary trials at this station (2) with lactating cows indicated that such a level approached the maximum amount of Aurofac 2A that could be fed in the grain mixture without affecting feed consumption, normal rumination, or palatability. The growth of dairy calves was enhanced when Aurofac was fed in amounts to supply 15 or 45 mg. of aureomycin per 100 lb. body weight daily (1). The level used in this experiment, the equivalent of 32 mg. of aureomycin per 100 lb. body weight daily, falls within this range.

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The grain mixture contained 500 parts of corn, 400 parts of wheat bran, 200 parts of soybean oil meal, 10 parts of bonemeal, and 15 parts of salt. This mixture was fed in sufficient amounts to meet nutrients allowances suggested in the Morrison standard (9) and was adjusted according to milk production at the start and the middle (end of 9th week) of the experiment. The roughage consisted of sorgo silage fed at the rate of 20 lb. daily per 1,000 lb. body weight and alfalfa hay of average quality fed *ad libitum*. All feed offered and refused was weighed and recorded.

The cows were kept stanchioned in the barn except for certain daily and nightly periods when they were allowed to exercise in a dry-lot. The cows had access to water from individual drinking cups in the barn. Since the feeding of antibiotics has been observed to increase the water consumption of dairy calves (2, 7), it seemed desirable to determine the effect of aureomycin on the water consumption of cows. Eight drinking cups had been fitted with water meters for a previous experiment. Four cows in each group (not paired) had their water intakes recorded. It was not possible to assign paired animals to stalls containing metered drinking cups because of variations in the sizes of the stalls and the animals. The cows were weighed for the first time on three consecutive days prior to the start of the preliminary period. They then were weighed on two consecutive days at fortnightly intervals, commencing two days prior to the start of period I. If the difference in the two consecutive weights for any animal was greater than 40 lb., the cows were weighed a third time, and this weight was included to obtain the average weight. The cows were milked twice daily. Fat tests were determined on 24-hour composite samples collected for the first time the day before the start of the preliminary period and thereafter at fortnightly intervals, commencing 1 day before the start of period I.

To determine whether the feeding of Aurofac 2A would affect normal rumination, the left flanks of the 18 experimental animals were palpated 5 and 2 days prior to the start of Aurofac 2A feeding and 2 and 7 days after the start of feeding Aurofac 2A (period I). The cows were always checked at the same time of day (1:30 to 3:00 P.M.), when the majority of them were in a resting-ruminating state. The movements were counted during 3-minute intervals and expressed as movements per minute.

On two occasions, during the 8th week of the experiment (period II) and during the 14th and 18th weeks (period III), pulse rates (palpation of ventral coccygeal artery) and body temperatures were measured on all animals to obtain some indication of the effect of Aurofac 2A feeding on the metabolic activity of the animals.

RESULTS

The feeding of Aurofac 2A appeared to be without effect on the general health and well-being of the animals. They remained in good health with the exception of cow 150A (group I), which developed mastitis during the 8th week of the experiment, and cow 290A (group II), which developed mastitis during the 14th week. These abnormal animals were maintained under experimental conditions for the duration of the trial, but their milk weights and

the milk weights of the cows paired with them, cows 128A and 287A, were excluded from the statistical analysis of the data.

Effect of Aurofac 2A on milk production. When the average pounds of milk produced by each group was plotted against time in days (not shown), the feeding of Aurofac 2A appeared to have no effect on milk production. Similarly when the average fat tests of each group were plotted against time in 2-week intervals, no effect ascribed to Aurofac 2A feeding was observed. The milk production data (Table 1) were analyzed by the method of Brandt (4) for reversal or switchback experiments. Since no carry-over effect due to Aurofac feeding was observed from graphical analysis, no allowance was made for a possible effect of transition between periods. Although in Table 1 it will be noted that the difference of 175.8 lb. of fat-corrected milk for 14 cows during an 18-week period is in favor of Aurofac 2A feeding, this difference amounted to only 0.15 lb. daily per cow. When treated statistically, this increase was not significant. The uncorrected milk production data were analyzed in a similar

TABLE 1

Effect of Aurofac 2A feeding [a] *on milk production*

Group	Cow No.	FCM produced			Difference [c]
		Period [b] I Control	Period [b] II Aurofac 2A	Period [b] III Control	I — (2 × II) + III
		(lb.)	*(lb.)*	*(lb.)*	
I	486A	705.5	661.9	568.9	—49.4
	162A	2,504.9	2,317.6	2,218.3	88.0
	178A	1,547.8	1,513.5	1,350.0	—129.2
	157A	730.9	601.2	396.0	—75.5
	471A	925.1	743.2	605.7	44.4
	175A	2,235.2	2,144.4	2,138.6	85.0
	253A	1,310.0	991.8	767.6	94.0
	Sums	9,959.4	8,973.6	8,045.1	57.3
	Av./cow/day	33.9	30.5	27.4	
		Aurofac 2A	Control	Aurofac 2A	
II	485A	873.0	845.0	775.2	—41.8
	171A	1,951.8	1,554.5	1,564.3	407.1
	186A	1,581.7	1,468.0	1,396.8	42.5
	160A	793.1	861.6	782.6	—147.5
	470A	900.0	890.8	792.7	—88.9
	173A	1,817.6	1,922.7	1,873.4	—154.4
	251A	2,213.0	1,988.3	1,979.7	216.1
	Sums	10,130.2	9,530.9	9,164.7	233.1
	Av./cow/day	34.5	32.4	31.2	

Total increase in milk production apparently attributable to Aurofac 2A feeding $= 233.1 - 57.3 = 175.8$ (lb.)

Av. increase per cow per day apparently attributable to Aurofac 2A feeding $= \frac{175.8}{2 \times 14 \times 42} = 0.15$ (lb.)

$t = 0.314$ $P > 0.7$

[a] Aurofac 2A included in the grain mixture at the daily rate of 40 g. per 1,000 lb. body wt.
[b] Each period of 6 wk. duration.
[c] Data analyzed by Brandt's method.

manner (data not presented). An increase of 0.14 lb. of milk for each cow each day as a possible result of Aurofac 2A feeding was obtained. This increase also was not statistically significant.

Effect of Aurofac 2A on feed consumption. The consumption of grain and silage did not appear to be affected by the feeding of Aurofac 2A. Only occasionally did refusals of grain or silage occur, and such refusals were not peculiar to either ration. Hay was fed *ad libitum*, as is customary in normal dairy practice, and, therefore, the chances of reflecting differences due to Aurofac 2A feeding were greater with hay feeding than with controlled grain or silage feeding. The feeding of Aurofac 2A apparently resulted in a total increased consumption of 433.9 lb. of hay for the 14 cows for 18 weeks (Table 2), or an average increase of 0.4 lb. for each cow each day. When analyzed by Brandt's method, this increase was not statistically significant.

When the hay consumption of the two mastitic cows (150A, 290A) and the cows paired to them (128A, 287A) was included (not shown), an average increase of 0.2 lb. more hay per cow per day resulted, apparently ascribed to Aurofac 2A feeding.

TABLE 2

Effect of Aurofac 2A feeding on alfalfa hay consumption [a]

Group	Cow No.	Alfalfa hay consumed			Difference
		Period I Control	Period II Aurofac 2A	Period III Control	I — (2 × II) + III
		(lb.)	*(lb.)*	*(lb.)*	
I	486A	780.5	768.7	627.7	—129.2
	162A	973.4	911.5	859.8	10.2
	178A	796.3	810.7	567.2	—257.9
	157A	950.3	884.0	952.1	134.4
	471A	652.5	549.5	494.4	47.9
	175A	800.7	868.0	708.5	—226.8
	253A	537.3	696.3	608.4	—246.9
	Sums	5,491.0	5,488.7	4,818.1	—668.3
	Av./cow/day	18.7	18.7	16.4	
		Aurofac 2A	Control	Aurofac 2A	
II	485A	622.1	678.1	562.2	—171.9
	171A	814.8	845.1	950.9	75.5
	186A	737.6	564.9	707.7	315.5
	160A	982.5	874.7	910.0	143.1
	470A	878.1	988.2	979.3	—119.0
	173A	1,011.9	1,052.7	879.2	—214.3
	251A	570.4	843.2	852.7	—263.3
	Sums	5,617.4	5,846.9	5,842.0	—234.4
	Av./cow/day	19.1	19.9	19.9	

Total increase in hay consumption apparently attributable to Aurofac 2A feeding $= 668.3 - 234.4 = 433.9$ (lb.)

Av. increase per cow per day apparently attributable to Aurofac 2A feeding $= \frac{433.9}{2 \times 14 \times 42} = 0.4$ (lb.)

$t = 0.662$ $P > 0.5$

[a] Footnotes similar to those in Table 1.

Effect of Aurofac 2A on water consumption. The data on consumption of water by the eight cows which had metered water bowls were analyzed according to Brandt's method and are presented in Table 3. The water consumption by the eight cows for the 18 weeks of experiment was 99.2 lbs. more while being fed unsupplemented rations, or an average consumption of 0.15 lb. less water per cow per day when the ration contained Aurofac 2A. This difference was not statistically significant.

Effect of Aurofac 2A on body weight. Throughout the experiment all animals

TABLE 3

Effect of Aurofac 2A feeding on water consumption [a]

Group	Cow No.	Water consumed			Difference I — (2 × II) + III
		Period I Control	Period II Aurofac 2A	Period III Control	
		(gal.)	*(gal.)*	*(gal.)*	
I	287A	623.5	585.9	535.4	—12.9
	157A	552.2	491.6	444.0	13.0
	175A	737.7	755.4	709.6	—63.5
	471A	464.6	387.4	343.7	33.5
	Sums	2,378.0	2,220.3	2,032.7	—29.9
	Av./cow/day	14.2	13.2	12.1	
		Aurofac 2A	Control	Aurofac 2A	
II	290A	546.2	579.5	491.2	—121.6
	186A	652.6	589.7	597.1	52.3
	128A	580.8	585.0	509.0	—80.2
	470A	620.0	516.7	433.8	20.4
	Sums	2,399.6	2,279.9	2,031.1	—129.1
	Av./cow/day	14.3	13.6	12.1	

Total decrease in water consumption apparently attributable to Aurofac 2A feeding $= 129.1 - 29.9 = 99.2$ (lb.)

Av. decrease per cow per day apparently attributable to Aurofac 2A feeding $= \frac{99.2}{2 \times 8 \times 42} = 0.15$ (lb.)

$t = 0.538$ $P > 0.5$

[a] Footnotes similar to those in Table 1.

remained in good state of flesh and body weight and did not appear to be adversely affected by the feeding of Aurofac 2A. During the period when Aurofac 2A was fed, the cows weighed an average of 7 lb. more than during the period when they received none. This difference was not statistically significant.

Effect of Aurofac 2A on rumination. The feeding of Aurofac 2A in amounts used in this experiment apparently had no effect on the number of rumen movements per minute or the strength of the movements (Table 4). The variation among animals for both measurements was exceedingly small (not shown). During the remainder of the experiment no deleterious effects on rumination as a result of Aurofac 2A feeding were observed.

Effect of Aurofac 2A on body temperature and pulse rate. The average pulse rates at selected times for the cows receiving Aurofac 2A for the four periods

TABLE 4

Effect of Aurofac 2A feeding [a] on frequency and strength of rumen movements

Period	Treatment		Rumen activity			
			Av. No. rumen movements per min.		Av. strength of movements [b]	
	Group I [c]	Group II [c]	Group I	Group II	Group I	Group II
Preliminary	No Aurofac 2A fed	5 days before feeding Aurofac 2A	1.6	2.1	2.9	2.8
Preliminary	No Aurofac 2A fed	2 days before feeding Aurofac 2A	1.8	1.8	2.4	2.9
Period I	No Aurofac 2A fed	2 days after start of Aurofac 2A feeding	1.7	1.8	3.0	3.2
Period II	No Aurofac 2A fed	7 days after start of Aurofac 2A feeding	2.1	2.0	3.1	3.3

[a] 40 g. per 1,000 lb. body wt. daily.
[b] Classification of strength of movements: quite weak, 1; weak, 2; medium, 3; strong, 4; quite strong, 5.
[c] 9 cows per group. Both groups checked on same days.

described in the experimental procedure were: 70.8, 63.8, 59.8, and 72.2. The corresponding average pulse rates for the cows not receiving Aurofac 2A were: 69.2, 65.3, 59.3, and 72.6. From these results, it would appear that the feeding of Aurofac 2A in amounts used in this experiment had no effect on pulse rate. The average pulse rates that were greater than 69 were obtained just prior to the afternoon feeding and milking. Similar observations were not made prior to the morning feeding and milking. The pulse rates lower than 69 were obtained at 10:00 A.M. (63.8 and 65.3) and at 8 P.M. (59.8 and 59.3), when the animals were resting. Body temperatures were taken concurrently with pulse rates. The average body temperatures for the cows receiving Aurofac 2A were: 101.1°, 100.6°, 100.9°, and 100.9° F. When not receiving Aurofac 2A, they averaged 101.1°, 100.8°, 100.7°, and 101.1° F. From these results it would appear that Aurofac 2A feeding does not affect body temperature.

DISCUSSION

Since initiation of this experiment, two preliminary reports on the feeding of antibiotics to lactating cows have appeared. Haq, Rusoff, and Gelpi (5) reported that the feeding of either 130 mg. of aureomycin, 130 mg. of tyrothricin, or 0.83 mg. of vitamin B-12 to Holstein cows (5 per group, plus 5 control animals) for a period of 60 days produced no detrimental effect on appetite and milk production. Loosli and Warner (8) fed six cows 100 mg. of aureomycin daily. During periods (duration not given) of antibiotic supplementation, these animals produced essentially the same amount of milk (FCM) as they produced during periods when no aureomycin was fed. No deleterious effects on feed consumption resulted. All these reports are in harmony with the results of the experiment herein reported. However, it remains to be determined whether or not significant increases in milk production with aureomycin feed-

ing can be obtained by using a lower plane of nutrition or by using a poorer type of roughage (e.g., non-legume).

SUMMARY AND CONCLUSIONS

A study, using 18 dairy cows, was made of the effect of feeding an aureomycin product (Lederle's Aurofac 2A) on milk and butterfat production, water consumption, body weight, rumination, pulse rate, body temperature, and general health and well-being. A double reversal design of feeding was used with three reversal periods of 6 weeks' duration. Aurofac 2A was fed at the daily rate of 40 g. per 1,000 lb. body weight. When Aurofac 2A was fed, the cows produced on an average 0.15 lb. more fat-corrected milk daily, consumed from 0.2 to 0.4 lb. more alfalfa hay daily, and 0.15 lb. less water daily than when no Aurofac 2A was fed. None of these differences were statistically significant. Body weight, rumination, grain and silage consumption, pulse rate, body temperature, and general health and well-being also were unaffected by Aurofac 2A feeding.

The results of this experiment seem to justify the conclusion that neither beneficial nor deleterious effects result from feeding lactating cows Aurofac 2A in amounts that supply the equivalent of 300 to 500 mg. of aureomycin daily per cow (based on body weight).

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THE HEAT RESISTANCE OF SOME LACTOBACILLI FOUND IN SWISS CHEESE [1]

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While many varieties of cheese have been made satisfactorily from pasteurized milk, the general feeling exists among Swiss cheese manufacturers that this cheese cannot be made successfully from pasteurized milk. This difficulty may be due to the destruction of microorganisms important in the ripening of the cheese and/or to a change in the physical structure or body of the cheese. Since lactobacilli have been found to be influential in the ripening process (1, 7, 8, 9, 10, 12, 13, 15), a knowledge of the heat resistance of these organisms should be helpful in approaching the problem of making Swiss cheese from pasteurized milk.

Bigelow and Esty (5) proposed a standard technique for determining the resistance of organisms to heat. Slatter and Halvorson (14) used minor modifications of this method in studying the heat resistance of lactobacilli found in cheddar cheese.

The object of this investigation was to characterize the thermal resistance of lactobacilli found in commercial Swiss cheese by determining the F and z values (2, 3) of a representative number of these organisms.

PROCEDURE

Twenty commercial U. S. Grade A and B Swiss cheeses varying in age from 6 weeks to 5 months were sampled for this investigation; ten cheeses were made in Wisconsin and ten in Ohio. The sampling was done in the Columbus market, taking approximately ¼ lb. samples of cheese directly to the laboratory.

Using aseptic methods, a 1-g. sample was obtained from the center of each ¼ lb. of cheese. This sample was ground to a homogenous suspension in 9 ml. of a 2 per cent sodium citrate solution using a sterile mortar and pestle. Further dilution for plating was obtained by using sterile water blanks. The plating was done on Difco tomato juice agar which was incubated at 37° C. until colonies appeared, the average time being from 48 to 72 hours.

Twenty-five contiguous colonies were picked from a representative plate having from 30 to 300 colonies. Five hundred colonies were picked from agar plates from the twenty cheeses used. The colonies were transferred into Difco reconstituted litmus milk (pH 6.4), which was incubated at 37° C. and the fermentation characteristics noted. If no change occurred in 2 weeks, the cultures were discarded. The Burke modification (6) of the Gram stain was used for the morphology work.

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The cultures selected for the study were all Gram-positive, non-spore-forming rods that produced acid in litmus milk or reduced and coagulated litmus milk with the formation of a smooth curd at 37° C. In order to be sure the cultures were pure, each one was streaked on tomato juice agar and incubated at 37° C., and single colonies again were transferred into litmus milk. Sixty cultures (three from each cheese) were selected for the heat resistance studies on the basis of their action on litmus milk and morphological characteristics. Each culture was carried through a series of six transfers in litmus milk before the heat studies were begun, to be sure the cultures were in an active, growing condition.

The method employed for the heat resistance studies was that of Bigelow and Esty (5) with minor modifications. The heating medium used was litmus milk, which was inoculated so that it would contain approximately 200,000 organisms per milliliter, as determined by direct microscopic count.

The inoculated litmus milk was aseptically dispensed into sterile Kimble brand No. 45050 chemical test tubes. These tubes were filled approximately half full with the litmus milk culture and sealed in a hot flame. The sealed tubes were submerged in a De Khotinsky, constant temperature water bath with a maximum temperature variation of $\pm$ 0.2° F. Five sealed tubes were removed at varying periods of time, the time being measured with a stop watch. In the determination of z values, ten tubes were removed at each time interval. The tubes were submerged immediately in water at room temperature. The tubes then were incubated for 4 weeks at 37° C. and observed for growth by noting changes in the litmus milk and by plating on tomato juice agar.

The heat lag was taken as 1 minute in all cases (11, 14).

The z values of nine selected cultures were determined, using the technique suggested by Ball (2, 3).

RESULTS

Three hundred twenty (64 per cent) of the 500 colonies selected from the 20 cheeses were rods. When inoculated into litmus milk, 227 cultures were found to be acid coagulating, 143 were acid noncoagulating, 58 peptonized the milk solids, and 72 were inert.

TABLE 1

A summary of thermal death times at 150° F. of 60 lactobacilli from swiss cheese

Time of exposure in minutes						
5	10	16	20	26	30	60
No. of cultures killed at each time interval						
4	22	24	5	2	1	2

A summary of the thermal death times at 150° F. of the 60 cultures is given in Table 1. Fifty of the 60 cultures selected were killed in 16 minutes or less at 150° F. Only two organisms were exceptionally heat resistant at this temperature. The plate count of the litmus milk heating medium ranged from 153,000 to 360,000 per milliliter, with the average being approximately 227,000.

TABLE 2

Thermal death times and z value of 9 selected lactobacilli

Culture numbers	Thermal death times in minutes at				z Values
	141° F.	144° F.	147° F.	150° F.	
34	50	22	12	3	8.0
27	35	13	5	2	8.5
59	29	13	5	2	8.0
15	70	22	8	3	7.0
39	35	16	9	5	11.0
46	60	30	14	9	10.5
28	40	20	10	5	9.5
53	90	40	20	8	8.5
11	....	90	55	35	14.0

The thermal death times at four different temperatures and the z values of nine selected cultures are given in Table 2. The z values vary from 7.0 to 14.0, with the least heat resistant organisms generally having the lower values and the more heat resistant organisms having the higher values.

Figure 1 shows the thermal death time curves of three cultures of varying heat resistance in comparison with the pasteurization curve. The pasteurization curve was constructed by passing a line through points representing 143° F. for 30 minutes (LTLT) and 160° F. for 18 seconds (HTST). The z value of this curve is 9. The path of the thermal death time curve of culture No. 15, z value 7.0, indicates that this organism could survive vat pasteurization, but not high-temperature, short-time treatment. Culture No. 28, z value 9.5, would be destroyed by vat pasteurization, but could survive high-temperature, short-time treatment. Culture No. 11, z value 14.0. could survive both types of pasteurization.

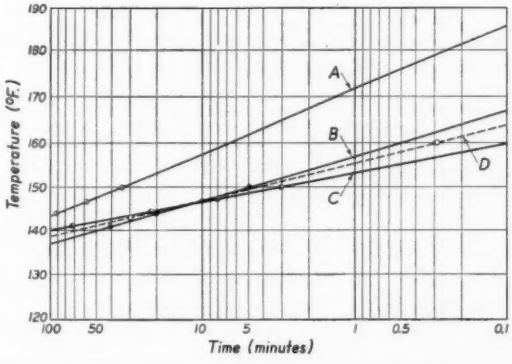

FIG. 1. A graphical comparison between the pasteurization curve and the thermal death time curves of three lactobacilli.

A Culture No. 11 z value 14.0
B Culture No. 28 z value 9.5
C Culture No. 15 z value 7.0
D Pasteurization curve (Minimum Standards)

DISCUSSION OF RESULTS

The heat resistance of lactobacilli found in Swiss cheese made from raw milk varied over a wide range, but the great majority of these organisms could withstand minimum pasteurization standards. The least heat-resistant organisms were killed in 5 minutes or less at 150° F., and, assuming a z value of 7, these results indicate that these organisms would be killed at 160° F. for 18 seconds. Only four of the 60 cultures fall in this group. The next most heat-resistant group could be killed in 10 minutes at 150° F. Assuming that this group of organisms had a z value of 7, a curve passing through 150° F. at 10 minutes shows that these organisms would survive the minimum standards for both types of pasteurization. Since only one organism studied had a z value as low as 7, the possibility of higher z values is quite evident. This would give an even greater assurance of this group of organisms surviving pasteurization.

Many cheese plants use pasteurization temperatures as high as 165° F. for 16 to 20 seconds. An organism having a heat resistance of 10 minutes at 150°F., z value 9.5, would be killed at 165° F. in 18 seconds. Twenty-five of the 60 organisms would be killed at this higher pasteurization temperature. Since this is such a large portion of the total number of lactobacilli, if this temperature is used, it probably would be advisable for the cheesemaker to adjust his manufacturing procedure to compensate for the destruction of such a large portion of the lactobacilli. This adjustment might be in the form of adding more starter and/or increasing the time of ripening the milk.

In a few instances where a heat treatment is used on milk for Swiss cheese manufacture, it is flash heated to 155° F. It is doubtful if this treatment kills any appreciable number of lactobacilli. It would require 155° F. for 24 seconds to kill the least heat-resistant organism studied.

The fact that some organisms could survive one type of pasteurization (HTST), but not the other (LTLT), illustrates that the two types of pasteurization are equivalent only for organisms with z values of 9.

SUMMARY AND CONCLUSIONS

The heat resistance of 60 lactobacillus cultures isolated from 20 commercial Swiss cheeses was studied by determining their thermal death times at 150° F. The thermal death times varied over a wide range, but 50 of the 60 cultures were killed in 16 minutes or less at 150° F. Only four (6.6 per cent) of the 60 cultures were killed at 160° F. in 18 seconds, whereas 25 (42 per cent) were killed at 165° F. in 18 seconds. Momentary heating at 155° F. would not kill an appreciable number of the lactobacilli found in commercial Swiss cheese. The most heat-resistant organisms had the highest z values, but the z values did not vary directly with the heat resistance in all cases.

If pasteurization exposures higher than the minimum are used, the destruction of lactobacilli at these higher temperatures suggests the possibility of adding more lactobacillus starter or lengthening the ripening time of the milk to facilitate the growth of the reduced numbers of lactobacilli that survive pasteurization.

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THE NUTRITION OF PSEUDOMONAS PUTREFACIENS
I. NITROGEN REQUIREMENTS

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Subsequent to the isolation and identification of *Pseudomonas putrefaciens* as the agent which causes the surface-taint defect of butter (2, 3), numerous investigators examined the factors affecting the appearance of this defect. Consequently, the importance of *Ps. putrefaciens* as a spoilage organism was established and the conditions influencing its development in butter were described (5). Few reports, however, have been concerned primarily with the nutritional requirements of this pseudomonad.

According to Long and Hammer (3), the growth of *Ps. putrefaciens* on beef infusion agar was not enhanced when this medium was supplemented with either alanine, asparagine, cysteine, cystine, glycine, or tryptophane. Wolochow *et al.* (6) observed that growth was obtained in the presence of either asparagine, creatine, casein, sodium caseinate, urea, ammonium sulfate, or dihydrogen ammonium phosphate. Potassium nitrate was not utilized by the organism nor was methyl or ethyl amine utilized.

The same authors (6) compared several complex media and reported that *Ps. putrefaciens* grew better on peptone-beef extract-agar, peptone-veal infusion-agar, and tryptone-glucose-beef extract-skimmilk-agar than on peptone agar or beef extract agar. In this connection, Claydon and Hammer (1) reported that casein agar, casein-peptone-agar, and casein-beef infusion-butterfat-agar were not superior to beef infusion agar as plating media. Long and Hammer (3) studied the growth response on numerous other complex media and from the results of these experiments devised the medium now commonly used for the isolation of *Ps. putrefaciens*.

Thus, it appears that the nitrogen requirements of *Ps. putrefaciens* are not clearly defined. It is the purpose of this paper to present data describing the growth response of this organism to amino acids, inorganic nitrogen salts, simple organic nitrogenous compounds, and complex organic nitrogenous materials.

METHODS AND MATERIALS

Cultures. The source of the cultures of *Ps. putrefaciens* used in this study is shown in Table 1. Cultures No. 1 through No. 7 were isolated and identified in this laboratory. The isolations were made by inoculating Long's agar (3) directly with water samples, or from water samples enriched in litmus milk. In addition to the usual identification procedures (3), the ability of each culture to produce the typical surface-taint defect in experimental churnings of butter

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TABLE 1

Sources of Ps. putrefaciens cultures

Culture No.	Source
1	Creek water, Illinois
2	Roadside water, Illinois
3	Roadside water, Illinois
4	Lake water, Illinois
5	Creek water, Illinois
6	Roadside water, Illinois
7	Drainage ditch water, Illinois
95	A.T.C.C. No. 8071, Hammer Strain 95
180	A.T.C.C. No. 8072, Hammer Strain 180
184	A.T.C.C. No. 8073, Hammer Strain 184

was determined. After repeated purification, the stock cultures were maintained by weekly transfers into sterile litmus milk.

Inocula. Inocula were prepared by streaking a portion of the stock cultures on slants of Long's agar. The growth resulting from 15 to 20 hours incubation at 21° C. was washed from the slants with 10 ml. of sterile distilled water. After centrifugation, the supernatant liquid was discarded and the packed cells were resuspended in 10 ml. of sterile water. This suspension was diluted with sterile water until the addition of one drop of inoculum to 10 ml. of medium did not cause an immediate change in optical density.

Media. Koser's citrate broth, containing 0.15 per cent sodium ammonium phosphate, 0.02 per cent magnesium sulfate, 0.3 per cent sodium citrate, and 0.1 per cent monopotassium phosphate, was used as the basal medium. This basal medium was modified when necessary, as noted in the text.

The amino acids and the concentrations in which they were employed in the first experiment are shown in Table 2. These concentrations represent the calculated levels and proportionality of the amino acids in the proteins of whole milk.

TABLE 2

Concentration of amino acids added to basal medium

Amino acid supplement	Concentration
	(γ/10 ml. basal)
1. DL-Alanine	400
2. L-Arginine	800
3. DL-Aspartic acid	1000
4. L-Cystine	100
5. L-Glutamic acid	2500
6. Glycine	100
7. L-Histidine	400
8. DL-Isoleucine	1000
9. L-Leucine	500
10. L-Lysine	1000
11. DL-Methionine	800
12. DL-Phenylalanine	1000
13. L-Proline	1000
14. DL-Serine	200
15. DL-Threonine	600
16. L-Tryptophane	300
17. L-Tyrosine	700
18. DL-Valine	1600

In other experiments, the amino acids were used in 0.0004 M concentrations, with and without the sodium ammonium phosphate component of the basal medium. Disodium phosphate was substituted for the ammonium salt in the latter instance. Similarly, the ammonium salt was omitted from the basal medium used to determine the growth response of *Ps. putrefaciens* to other nitrogen sources. In these studies, the nitrogenous compounds or mixtures were used in 0.1 per cent concentrations.

The media were adjusted to pH 7.0, dispensed in 10-ml. quantities into optically standardized 18 × 150 mm. culture tubes, and sterilized at 121° C. for 20 minutes. The nitrogenous compounds susceptible to decomposition by heat were sterilized by the use of diethyl ether. In these instances the nitrogenous material was placed in a sterile culture tube and flooded with ether. The cotton stoppers were saturated with ether and then the tubes and their contents were permitted to stand undisturbed until the ether had evaporated. Ether was used in such quantities that it did not evaporate before an elapsed time of 48 hours. Materials sterilized by this means were examined for sterility before they were added aseptically in calculated amounts to sterile basal medium.

After inoculation with one drop of standardized inoculum, the test media were incubated at 21° C. An incubation period of 14 days was employed in the amino acid utilization studies. A 5-day incubation period was used in the other experiments.

Growth. Growth in the synthetic media containing amino acids was measured turbidimetrically in a model 400 Lumetron photoelectric colorimeter equipped with a No. 370 filter and recorded as optical density × 10. A No. 420 filter was used to estimate growth in the other experimental media.

On completion of the prescribed incubation period, one drop of each mature culture was inoculated into an identical tube of sterile medium, which then was incubated for the same period. This process was repeated again to obtain a third reading. The experimental data are expressed as optical density × 10 and represent the mean of three observations made in the above fashion. The data obtained in each experiment were subjected to an analysis of variance (4) in order to assess the relative importance of the sources of variation.

RESULTS

The growth response of ten cultures of *Ps. putrefaciens* to 18 single amino acids added to the basal medium containing 1 mg. of sodium ammonium phosphate per 10 ml. is shown in Table 3. The amino acids were used in the concentrations given in Table 2. These data show that only one of the ten cultures was able to grow in the inorganic nitrogen basal medium. The addition of alanine, aspartic acid, glutamic acid, isoleucine, leucine, serine, tryptophane, or tyrosine to the basal medium materially increased the growth of this culture. Tryptophane and tyrosine were especially effective in this regard. The remaining nine cultures were unable to grow in the basal medium containing sodium ammonium phosphate as the sole source of nitrogen. When certain amino acids were added, detectable growth occurred in some instances.

TABLE 3

Utilization of amino acids by Ps. putrefaciens when supplemented by inorganic nitrogen

(mean of 3 determinations expressed as O.D. × 10)

Amino Acid Added [a]	Culture No.									
	1	2	3	4	5	6	7	95	180	184
DL-Alanine	—	—	0.13	—	—	—	—	—	1.64	—
L-Arginine	—	—	0.28	—	—	—	—	0.07	1.37	—
DL-Aspartic acid	0.08	0.16	0.22	0.07	0.03	0.11	0.01	0.72	1.61	0.13
L-Cystine	—	—	—	—	—	—	—	—	1.38	—
L-Glutamic acid	0.49	0.46	0.66	0.39	0.26	0.65	0.46	0.52	1.63	0.36
Glycine	—	—	0.04	—	—	—	—	—	1.32	—
L-Histidine	—	—	—	—	—	—	—	—	1.53	—
DL-Isoleucine	0.15	0.18	0.31	0.12	0.19	0.24	0.16	0.18	1.63	0.16
L-Leucine	—	0.17	0.64	0.10	0.13	0.22	0.15	0.16	1.77	0.14
L-Lysine	—	—	—	—	—	—	—	—	1.51	—
DL-Methionine	—	—	0.13	—	—	—	—	—	1.46	—
DL-Phenylalanine	—	—	0.10	—	—	—	—	—	1.59	—
L-Proline	—	—	0.18	—	—	—	—	0.29	1.53	0.24
DL-Serine	—	—	0.05	—	—	—	—	—	1.62	0.20
DL-Threonine	—	0.01	0.24	—	—	0.10	—	0.03	1.44	—
L-Tryptophane	—	—	0.13	0.77	—	—	—	—	1.99	—
L-Tyrosine	0.13	0.25	0.46	0.19	0.17	0.30	0.22	0.20	1.90	0.20
DL-Valine	—	—	0.07	—	—	—	—	0.18	1.51	0.16
None	—	—	—	—	—	—	—	—	1.41	—

[a] In concentrations shown in Table 2. Incubated at 21° C. for 14 days. — = no growth.

Thus, the combination of inorganic nitrogen and glutamic acid permitted growth of all ten cultures. Under the same conditions, nine cultures responded to isoleucine or tyrosine and seven to leucine. With the exception of culture No. 180, the test cultures did not grow when cystine, glycine, lysine, histidine, methionine, alanine, and phenylalanine were added to the basal medium. Analysis of these data indicated that highly significant differences in the amounts of growth obtained could be attributed to both cultures and substrates. There were no significant differences in the amounts of growth obtained in the three successive trials.

In general, the growth pattern shown in Table 3 was not changed greatly when the amino acids were tested in 0.0004 M concentrations in the presence of sodium ammonium phosphate.

When the amino acids were used as the sole source of nitrogen in the medium, the extent of growth was less than that encountered in the two previous experiments. This effect was particularly evident in the response of culture No. 180. However, ten cultures grew in isoleucine, nine in tyrosine, eight in glutamic acid, and six in leucine media.

With the exception of culture No. 180, which grew in all of the amino acid media, the test cultures exhibited a doubtful or negative growth response in media containing either arginine, aspartic acid, cystine, glycine, histidine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, or valine. These results are shown in Table 4. In this experiment it was noted that highly significant differences in the growth data could be attributed to the subculturing procedure. Additional growth was obtained upon subculturing into the identical

TABLE 4

Utilization of amino acids by Ps. putrefaciens

(mean of 3 determinations expressed as O.D. × 10)

Amino Acid Added [a]	Culture No.									
	1	2	3	4	5	6	7	95	180	184
DL-Alanine	—	0.01	—	0.01	0.07	0.09	0.06	0.15	0.31	0.03
L-Arginine	—	—	—	—	—	—	—	—	0.76	—
DL-Aspartic acid	0.10	0.05	0.10	0.08	0.07	0.10	0.01	0.10	0.25	0.13
L-Cystine	—	—	—	—	—	0.01	—	—	0.17	—
L-Glutamic acid	0.23	0.14	0.18	0.14	0.08	0.18	0.07	0.21	0.31	0.21
Glycine	—	—	—	—	—	—	—	—	0.23	—
L-Histidine	—	—	—	—	—	—	—	—	0.27	0.04
DL-Isoleucine	0.23	0.14	0.19	0.15	0.16	0.31	0.21	0.25	0.31	0.19
L-Leucine	—	0.13	0.21	0.13	0.16	0.21	0.19	0.18	0.22	0.08
L-Lysine	—	—	—	—	—	—	—	—	0.23	—
DL-Methionine	—	—	—	—	—	—	—	—	0.34	—
DL-Phenylalanine	—	—	—	—	—	—	—	—	0.22	—
L-Proline	—	—	—	—	—	—	—	0.07	0.25	—
DL-Serine	0.01	0.06	0.08	0.04	—	0.06	—	0.07	0.23	0.05
DL-Threonine	—	0.01	—	0.03	0.07	0.09	0.04	0.10	0.16	0.03
L-Tryptophane	—	—	—	—	—	—	—	—	0.41	—
L-Tyrosine	0.25	0.10	0.16	0.29	0.16	0.29	0.21	0.15	0.29	0.15
DL-Valine	—	—	—	—	—	—	—	0.09	0.22	0.07
None	—	—	—	—	—	—	—	—	—	—

[a] 0.0004 M concentration of the l form. Incubated at 21° C. for 14 days. — = no growth.

media. This was especially true in the case of the amino acids utilized by the majority of the cultures. Also, upon subculturing, detectable growth occasionally developed in the presence of amino acids not previously utilized. Thus it appears that several of these cultures can be adapted to utilize certain amino acids.

These data indicate that different cultures of *Ps. putrefaciens* vary considerably in their growth response to selected amino acids as the source of nitrogen in a minimal medium. Under such conditions amino acids supported only slight to moderate amounts of growth.

Similar results were observed when the test cultures were grown in media containing ammonium salts as the sole source of nitrogen. *Ps. putrefaciens* No. 180 grew in the presence of 0.1 per cent concentrations of ammonium chloride, ammonium nitrate, dibasic ammonium phosphate, ammonium sulfate, ferric ammonium citrate, or sodium ammonium phosphate. Meager growth was established by culture No. 184 in dibasic ammonium phosphate medium, whereas the remaining cultures did not respond to any of the ammonium salts.

When asparagine was tested as the nitrogen source in minimal basal medium, eight cultures established moderate growth and two cultures grew meagerly. With the exception of creatine and creatinine, which supported only scant growth of culture No. 180, adenine sulfate, creatine, creatinine, guanine, uracil, urea, uric acid and xanthine did not permit the growth of the *Ps. putrefaciens* cultures.

In contrast to the foregoing data, many complex organic nitrogen sources were readily utilized by the test cultures. As shown in Table 5, protein hydrolysates gave rise to moderate levels of growth by the majority of the test cultures. Maximum amounts of growth were observed in the media containing protolysate, casitone, casamino acids, or tryptone. While other materials, such as sodium

TABLE 5

Utilization of complex organic nitrogen substrates by Ps. putrefaciens

(mean of 3 determinations expressed as O.D. × 10)

Additions to basal medium	Culture No.									
	1	2	3	4	5	6	7	95	180	184
Albumin, egg [a]	0.11	0.32	0.06	0.12	—	0.53	0.04	0.62	0.45	0.13
Beef [b]	0.21	0.21	0.14	0.22	0.21	0.57	0.62	0.64	0.15	0.14
Beef extract [b]	0.52	0.41	0.54	0.48	0.46	0.67	0.71	0.88	0.32	0.43
Casamino acids [b]	1.92	1.94	1.34	1.47	1.38	1.42	1.96	2.02	0.76	1.81
Casein [b]	0.08	0.20	0.01	0.13	0.42	0.93	1.48	0.13	0.07	0.13
Casein, isoelectric [b]	—	0.07	—	0.05	0.10	0.01	0.48	0.13	0.11	0.01
Caseinate, sodium [b]	0.15	0.07	0.10	0.22	0.30	0.15	0.18	0.13	0.14	0.27
Casitone [b]	1.94	2.20	1.66	2.02	2.20	1.75	1.79	2.20	0.48	1.92
Gelatin [b]	0.04	0.11	0.08	0.15	0.59	1.43	1.43	1.51	0.59	0.13
Gluten [c]	0.03	0.16	0.11	0.13	0.68	0.67	1.18	0.79	0.62	0.35
Lactalbumin [c]	0.50	1.13	1.14	0.21	0.90	0.15	0.74	1.59	0.40	1.18
Liver [b]	0.24	0.53	0.15	0.27	0.42	0.45	0.81	0.33	0.01	0.16
Milk, peptonized [b]	0.87	1.10	1.10	0.92	1.10	1.14	0.92	0.98	0.11	0.92
Pancreatin [d]	0.07	0.20	0.21	0.13	0.27	0.28	0.21	0.33	0.12	0.19
Pepsin [d]	1.32	1.28	1.29	1.35	1.21	1.18	1.78	1.92	0.01	1.37
Peptone [b]	1.53	1.70	1.42	1.49	1.55	1.64	1.49	1.72	0.49	1.90
Peptone [c]	1.49	1.87	1.64	1.64	1.17	1.66	1.89	1.68	0.53	1.65
Peptone [f]	1.32	1.99	2.00	1.71	0.94	1.53	2.63	2.03	—	1.72
Proteose-peptone [b]	0.76	1.10	0.99	0.96	1.24	1.27	1.37	1.53	0.10	1.52
Proteose-peptone No. 2 [b]	1.39	1.29	1.25	1.43	1.27	1.58	1.63	1.92	0.45	1.63
Protolysate [g]	1.85	1.92	1.81	2.06	2.27	1.93	2.26	2.00	0.25	1.92
Tryptone [b]	1.67	1.47	1.59	1.71	2.05	1.57	1.83	1.97	0.17	1.90
Tryptose [b]	0.62	1.03	0.81	1.43	1.39	1.12	1.16	1.69	0.06	1.50
Yeast, autolyzed [b]	0.61	0.56	0.38	0.40	0.99	0.62	0.25	0.17	—	0.44
Zein [c]	—	0.04	0.04	—	0.05	0.11	0.07	0.19	0.26	0.11
None	—	—	—	—	—	—	—	—	—	—

[a] Mallinckrodt
[b] Difco
[c] General Biochemicals
[d] Merck
[e] Wilson
[f] Witte Peptonum Siccum
[g] Mead-Johnson

— = no growth.

caseinate and tryptose, were utilized by all ten cultures, growth in these media was less than in the above-mentioned four media.

Some of the relatively intact protein materials, such as casein, isoelectric casein, egg albumin, and zein, were utilized by only a few of the test organisms to a limited extent. Other proteins of this category, such as lactalbumin, gelatin and glutin, which did promote growth, were less effective in this regard than the hydrolyzed proteins.

The poor growth response of culture No. 180 to the complex nitrogenous materials used in this experiment is in contrast to the growth of this organism on the media containing ammonium salts as a source of nitrogen.

DISCUSSION

Long and Hammer (3) observed wide variation between cultures of *Ps. putrefaciens* but they considered these variations too insignificant to justify varietal designations. They regarded the amount of growth as a factor in variation and stated that some cultures are difficult to cultivate on artificial media. The same authors (3) pointed out that even when growth is once established,

the development may not be extensive in some cultures and as a consequence the changes produced in various media may differ from those produced by vigorous cultures.

The foregoing data illustrate some of the variation encountered in the response of cultures of *Ps. putrefaciens* to various materials employed as sources of nitrogen in media. These data suggest that the variation between cultures of *Ps. putrefaciens* may be the cause of limited development of some cultures on artificial media. The culture variations emphasize the problem of formulating a satisfactory medium for the isolation of *Ps. putrefaciens*. Furthermore, basic differences in cultures may be related to unsuccessful attempts to isolate *Ps. putrefaciens* from putrid butter. This may be especially true when the numbers of viable organisms in a sample are low.

In general, the data show that inorganic nitrogen, amino acids, and simple organic nitrogenous compounds were less suitable than complex hydrolyzed proteins for the cultivation of a majority of *Ps. putrefaciens* cultures. However, there apparently exist some strains of this organism, as exemplified by *Ps. putrefaciens* No. 180 (ATCC 8072, Hammer strain 180), which are capable of attaining good growth in media containing inorganic nitrogen sources. While this strain grew also in the presence of any one of 18 amino acids employed as a sole source of nitrogen, the amount of growth was less than that in the inorganic nitrogen medium. The same organism grew relatively poorly on the hydrolyzed protein materials.

Ps. putrefaciens 180 established growth when any one of 18 amino acids was employed as the nitrogen source in a synthetic medium, but the remaining strains of the test organism were less versatile in this regard, and growth was obtained only in the presence of certain amino acids, such as aspartic acid, glutamic acid, isoleucine, leucine and tyrosine. The amino acids cystine, glycine, histidine and lysine, when tested in both the presence and absence of ammonium salts, failed to support growth of nine of the ten cultures.

SUMMARY

Nine of ten cultures of *Ps. putrefaciens* failed to grow in media containing ammonium salts as a source of nitrogen. Certain amino acids, asparagine, numerous complex hydrolyzed proteins, and intact protein materials were utilized. Hydrolyzed proteins afforded the best growth of the test cultures.

One culture of *Ps. putrefaciens* which utilized ammonium salts, amino acids, and asparagine as a source of nitrogen grew relatively poorly in complex organic nitrogenous materials.

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THE RELATION OF CERTAIN SEMEN-QUALITY TESTS TO BREEDING EFFICIENCY AND CHARACTERISTICS OF SEMEN FROM LOW-FERTILITY BULLS BEFORE AND AFTER HORMONE INJECTION

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I. THE RELATION OF CERTAIN SEMEN-QUALITY TESTS TO BREEDING EFFICIENCY

A number of laboratory tests have been devised to determine the fertility of bulls. Williams and Savage (12) found a relation between motility, concentration, percentage of abnormal forms, and head length of spermatozoa in bulls of good, average, and poor breeding efficiency. To determine semen quality, Beck and Salisbury (1) reported the use of methylene-blue reduction time; Mann (9) suggested an index based on utilization of fructose; and Lasley, Easley, and McKenzie (8) suggested a stain for differentiation between living and dead spermatozoa. Herman and Swanson (6) reported the time of survival with good motility to be a good index of fertility. These authors also reported that semen from bulls of good, questionable, and poor fertility averaged 14.2, 24, and 45.8 per cent of abnormal forms. Herman *et al.* (6) and Williams *et al.* (12) point out that a low percentage of abnormal forms is not a guarantee of high fertility.

Several workers have related these semen tests to fertility. Swanson and Herman (11) found a correlation of + 0.84 between the viability of the sperm in storage and their ability to produce pregnancy. They also found a curvilinear relation between motility and conception rate. This latter relation disappeared when samples with a motility rating of three or more were used. Buckner and Willett (3) calculated multiple correlation coefficients for a group of laboratory tests and conception. They concluded that a combination of tests gave values high enough to enable fairly accurate prediction of fertility. Salisbury (10) reported a correlation coefficient between methylene-blue reduction time and conception rate ranging from — 0.5 to — 0.7. Hill and Gassner (7) found a relation between the average fructolysis index and breeding efficiency of 17 bulls ($r = 0.83$) computed on a 60-90 day non-return.

EXPERIMENTAL PROCEDURE

Semen samples obtained from normal fertile bulls in the artificial insemination stud of the California Dairy Breeders, Inc., were tested for motility, concentration, percentage dead, fructolysis index, and percentage abnormal sperm. Tests were run only on the samples that were shipped from the stud. Semen is not used by this breeding association unless motility is 60 per cent or

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more. Breeding efficiency for this study was calculated on the basis of 30-60 day non-return from first and second services. Most of the breedings occurred within 48 hours after the collection of semen. Fifty-five samples from 12 bulls and 1,010 breedings were used in the study. Breeding efficiency for individual semen samples ranged from 87 to 55 per cent.

The fructolysis index was measured after the method of Mann (9), using semen in a phosphate buffer containing antibiotics in the same concentration as was used in the semen diluent. The number of live and dead sperm and abnormal forms were counted from slides prepared according to the procedure outlined by Lasley *et al.* (8). In our experience abnormal forms counted from these slides show from zero to 8 per cent less than when counted from slides stained by the alkaline methyl-violet method of Blom (2). Concentration was determined by counting in a hemocytometer, and motility was estimated by the stud manager.

II. CHARACTERISTICS OF SEMEN FROM LOW-FERTILITY BULLS BEFORE AND AFTER HORMONE INJECTION

Gregory *et al.* (5) reported the occurrence of a simple, autosomal, recessive gene for low fertility in Holstein bulls. The semen produced by these bulls was characterized by a low concentration of spermatozoa and a high percentage of abnormal forms. That this condition might be due to certain hormonal insufficiency or imbalance led to the initiation of the following investigation.

EXPERIMENTAL PROCEDURE

The study involved three inbred Holstein bulls 1, 2, and 3 (coefficient of inbreeding—25 per cent) that were related to those reported by Gregory *et al.* (5). Semen from these bulls resembled the semen from the low-fertility bulls reported by the above authors, being low in concentration and high in percentage of abnormal forms.

Each bull acted as his own control, since samples of semen were collected before and after hormone treatment. The procedure was as follows.

Several weekly samples were collected from each bull before starting hormone treatment. These samples, together with weekly collections made for 2 weeks following the first hormone injection and beginning again 3 weeks or more after treatment ceased, were used as the controls. Samples collected the third week after hormone treatment was initiated and weekly throughout the treatment period, as well as for 2 weeks after hormone injections ceased, were used to determine the effect of the hormones on semen characteristics. This procedure has been followed since Gassner and Hill (4) have reported that, following treatment of bulls with estrogen, the semen quality was destroyed by the third week. The gametogenic tissue was affected first. They state that recovery occurred in the same order, but do not report time required for recovery.

Since none of the hormone treatments here reported brought the semen back to normal in all respects, the hormones were injected at one level until the char-

acteristics of the semen appeared to be stabilized, at which time the dosage was doubled. All hormone preparations were suspended in a 0.9-per cent saline solution and injected subcutaneously.

Bulls 1 and 2 were injected with a gonadotrophin [1] from horse pituitary, high in follicle-stimulating hormone, at the rate of 1,000 r.u. thrice weekly for 4 weeks, followed by daily injections of 1,000 r.u. of the same hormone preparation for another 4 weeks. During the next 3 weeks they received weekly injections of 8,000 i.u. of pregnant mares' serum. Since the pituitary gonadotrophin and the P.M.S. showed the same effect on the semen, the results are grouped together in Table 1.

TABLE 1

Characteristics of semen from low-fertility bulls before and after treatment with certain endocrine preparations compared with that from normal untreated bulls

Quality tests	Bull 1 [a]		Bull 2 [a]		Bull 3 [b]		Bull 4	Bull 5
	Control [c]	Experimental	Control [c]	Experimental	Control [c]	Experimental	Control	Control
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Concentration sperm /cmm./10,000	69	112.7 [d]	66.7	92.7	39.1	74.4 [d]	85.14	123.3
Percentage live	41	33	56.5	60.8	20.4	30.0	71.4	79
Percentage abnormal	41	47.7	19	15.9	43.7	43.2	6.35	10
Fructolysis index	0.716	1.75	2.43	2.75	.....	.....	2.40	.....
Fructose mg./100 ml.	686	591	771	719	.....	.....	502	.....
Motility percentage	12	10	37	41	7	11	49	66

[a] Bulls 1 and 2 were injected with gonadotrophins.
[b] Bull 3 was injected with lyophilized beef anterior pituitary.
Bulls 4 and 5 were normal untreated bulls.
[c] Each experimental bull served as his own control.
[d] Significant at .01 level.

Bull 3 was injected daily with 100 mg. of lyophilized beef anterior pituitary [2] for 5 weeks, followed by daily injection of 200 mg. for another 5 weeks. Semen samples were collected from normal untreated bulls 4 and 5 during the experimental periods as a check on environmental factors. Bull 4 served as a control for experimental bulls 1 and 2, and bull 5 as the control for experimental bull 3.

RESULTS AND DISCUSSION

Simple correlations between semen-quality tests and conception rate were as follows: motility, $r = 0.015$; concentration, $r = 0.078$; percentage dead, $r = -0.51$; fructolysis index, $r = -0.018$; percentage abnormal sperm, $r = -0.83$. Percentage dead and percentage abnormal are both significant at $P = 0.01$.

All the tests show a large variation. The least variable of these tests were percentage abnormal forms and percentage dead. The fructolysis index varied widely among samples of semen. Samples with a high concentration of sperm

[1] Courtesy of Searle Laboratories.

[2] Courtesy of Armour Research Laboratories.

used practically all of the available sugar the first hour; hence the second hour the index was very low. The test was calculated on a 2-hour basis, so that a low index was recorded from samples of semen which were of good fertility but contained large numbers of sperm and a low concentration of fructose. Conversely, a sample containing few sperm of good motility and a high concentration of sugar gave high values for the index. In the normal bulls, the index ranged from 1.9 to 5.00, averaging 2.74.

Data on the bulls of low fertility of genetic origin and control bulls are presented in Table 1. Bulls 1 and 2 were injected with the gonadotrophins, and bull 4 served as their normal control. Bull 3 was injected with the lyophilized pituitary, and bull 5 was used for the normal control.

Injections of the gonadotrophins and whole pituitary increased the concentration of spermatozoa in all three bulls. In two of the three individuals, this increase was significant. None of the other characteristics of the semen were changed significantly.

Fructose determinations on two of the bulls (1 and 2) indicated a normal amount of fructose in the semen. The rate of fructose utilization by the semen from one bull was within the normal range even in the presence of large numbers of dead and abnormal sperm. The increase in utilization of fructose by the semen from bull 1 during the experimental period was not significant. The normal amounts of fructose indicated a normal secretion of testosterone. The bulls were normal with respect to their secondary sex characters, and this also implied a normal secretion of testosterone. The secretion of normal amounts of testosterone indicates a normal secretion of the luteinizing hormone.

It appears that one factor involved in the production of poor-quality semen by these bulls was a deficiency of the follicle-stimulating hormone, as indicated by the increase in sperm concentration following treatment with gonadotrophin. However, a deficiency of follicle-stimulating hormone was not the only factor involved since this hormone did not correct all of the abnormalities. Involvement of other factors is also indicated because doubling the amount of the hormones showed no improvement over the original amount given. If the original quantities were not adequate to produce the full effect of the hormone, the increased dosage should have given a greater response.

Whether increasing the length of the period of treatment would have caused further change in the semen characteristics was not determined. The increase in concentration of spermatozoa following treatment and return to pre-treatment levels upon cessation of treatment indicates that the time periods were adequate. Further work needs to be done to determine the length of time required for the spermatogenic cycle in the bull.

SUMMARY

Laboratory tests for semen quality including concentration, motility, fructolysis index, percentage dead sperm, and percentage abnormal sperm were investigated for their relationship to breeding efficiency. The two latter tests were most closely correlated to fertility, as measured by 30-60 day non-returns.

A deficiency of follicle-stimulating hormone appears as one of the factors involved in the production of low-quality semen by certain related bulls.

The luteinizing hormone and testosterone appeared to be secreted in normal amounts in these bulls.

Factors other than the above are indicated to have been involved in poor-quality semen production by three inbred Holstein bulls.

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THE EFFECT OF HEAT TREATMENT ON THE SULFHYDRYL GROUPS IN SKIMMILK AND NONFAT DRY MILK

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At the present time, it is generally believed that cystine and cysteine are responsible for all the disulfide and sulfhydryl groups of proteins. Although a certain amount of evidence has appeared that this statement may not always be true (11), the experiments to be described on milk were carried out on the basis of this hypothesis. It is generally assumed also that the equilibrium between cystine and cysteine in a protein can be shifted with relative ease although in so doing the chemical, physical, and biological properties of the protein may be markedly affected. Thus, the protein, pre-keratin, is soluble and readily digestible and contains primarily cysteine. When the cysteine is oxidized *in vivo* to cystine, the insoluble, indigestible protein, keratin, is formed. On the other hand, the hormone, insulin, is devoid of cysteine but contains a very sizable quantity of cystine. If only a very small proportion of the cystine is reduced to cysteine, the biological effects of insulin are lost (14).

Milk proteins, specifically the β-lactoglobulin fraction (3), also contain both cystine and cysteine. Numerous studies have shown that the presence of the sulfhydryl groups in milk, presumably all present in the proteins as cysteine, have a very marked effect on the flavor of fluid and dried milk, on the keeping qualities of evaporated and dried milk, and on other properties (1, 13, 15). The supposed deleterious effect of the -SH groups of β-lactoglobulin on the baking qualities of bread dough made with improperly heated milk or milk products has been questioned by the observation that unheated β-lactoglobulin does not cause poor loaf formation (7). The "cooked flavor" of heated milk has been associated with the decomposition of the -SH groups, possibly to H_2S (5).

The object of this study was to ascertain, if possible, the relationship between the appearance of the "cooked flavor" and the change in sulfhydryl groups on heating. Although considerable work had been carried out on this problem prior to our experiments, it was believed that the methods used for the estimation of the sulfhydryl groups were either nonspecific, very laborious, or required equipment not usually available in the laboratories of milk plants (4, 7, 8, 9, 12, 13). The introduction by Kolthoff (6) of a specific amperometric titration method for -SH groups and its adaptation to these groups in proteins by Benesch (2) appeared to offer a solution of these difficulties. Since our experiments were undertaken, Hutton and Patton (5) have published their results on milk, using the Kolthoff procedure. However, as the results of Hutton and Patton and ours do not agree in all respects, it was thought desirable to publish the present report.

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EXPERIMENTAL

Theory. The amperometric titration of mercaptans (sulfhydryl groups) is based on the fact that when silver ions react with -SH compounds, an insoluble, very slightly dissociated product results. As long as free -SH groups exist in solution or in fine suspension, there will be only a small amount of silver ions in solution. However, when all the -SH groups have been converted to the silver mercaptides, the further addition of the soluble silver salt will result in the rapid increase of Ag^+ ions in solution. This latter increase of Ag^+ ions measured amperometrically, is the end-point of the reaction.

Apparatus. The equipment used is essentially that of Kolthoff (6). It consists of a rotating platinum electrode, a reference electrode, a galvanometer and an automatic burette (Figure 1). The only change from the apparatus described

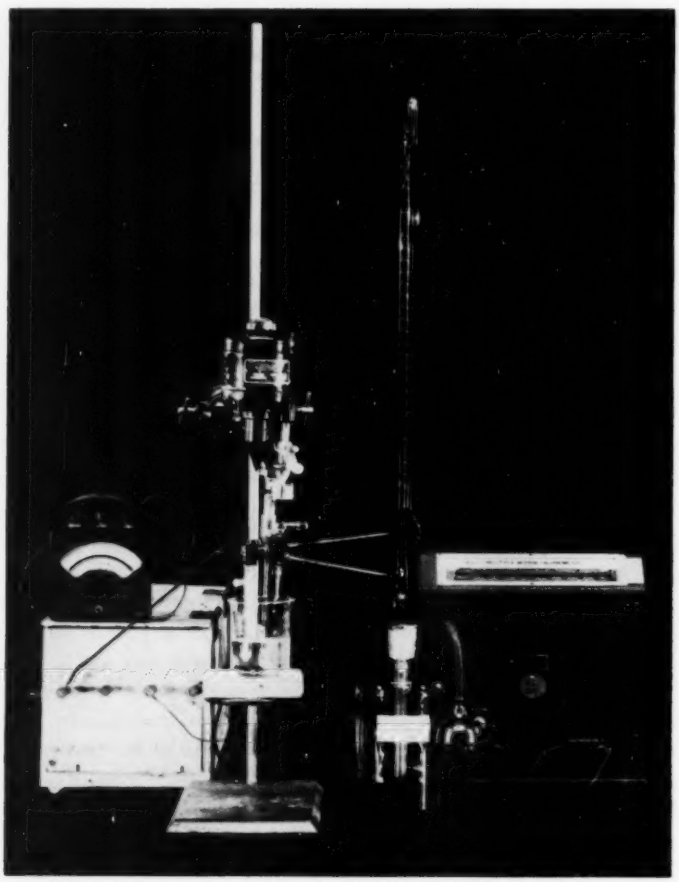


FIG. 1. Apparatus for -SH group analysis.

by Kolthoff is that a commercially available calomel electrode (Beckman No. 1170) is substituted for the external HgI_2 reference electrode. An external potential of –0.15 V is applied by means of a dry cell.

Titration. One hundred ml. of 0.1 M sodium acetate and 1.0 ml. of ammonium hydroxide (sp. gr. 0.90) are mixed in a 250-ml. beaker.[1] The use of sodium acetate, in place of NH_4NO_3 or NH_4NO_3–C_2H_5OH (2, 5, 6), resulted in the titration of the -SH groups in milk proteins. A 25.0-ml. sample of skimmilk or 25.0 ml. of reconstituted skimmilk is added to the solution. A stream of nitrogen is then passed through the solution for 5 minutes at a rate to minimize foaming and surface denaturation. The gas is dispersed by a sintered glass bubbler. The contents of the beaker are titrated with 0.001 *N* $AgNO_3$ in the apparatus described above.

In contrast to simple, soluble mercaptans such as cysteine, the milk protein titrations showed a sharp increase in diffusion current prior to the end-point, followed by a slow drift towards zero diffusion current.[2] This phenomenon continued until the end-point was passed, after which the diffusion current (galvanometer readings) increased proportionally to the amount of Ag^+ ions in solution.

Because of the lack of a sharp end-point, the following procedure was employed. Silver nitrate was added rapidly to the reaction vessel until a small excess was present, as indicated by the deflection of the galvanometer needle. Then 1.0-ml. aliquots of $AgNO_3$ solution were added, each spaced 60 seconds apart. The galvanometer responses were noted just before each successive addition. In this way a series of points could be obtained. A straight line drawn through four or five of these points plotted on graph paper and extrapolated to zero diffusion current gave accurate, reproducible end-points. A typical titration curve is shown in Figure 2.

Milk samples. Fresh, raw whole milk was stored in the refrigerator for one day, and then the cream was removed. Nonfat dry milk (vacuum roll or spray dried) was furnished by D. E. Mook, New Products Research Laboratory, The Borden Company, N. Y. Whey was prepared from raw skimmed milk by precipitating the casein with acetic acid at pH 4.55. Protein-free whey was made by adding sufficient 50 per cent trichloracetic acid to the whey until the concentration of T.C.A. reached 10 per cent. The filtrate from this T.C.A. precipitation was called "protein-free whey."

The milk samples were heated without agitation in 500-ml. round bottom flasks immersed in a thermostatically controlled water bath.[3] The heating was

[1] The addition of a large amount of ammonium hydroxide makes the titration feasible in the presence of chloride or bromide ions (6). The reaction in the presence of ammonia proceeds as follows:

$$Ag(NH_3)_2^+ + RSH \rightarrow RSAg + NH_4^+ + NH_3$$

[2] This drift in the diffusion current, which is encountered in the case of protein but not in the case of titrations of soluble mercaptans, may be explained on the basis that an appreciable time is required for the Ag^+ ions to penetrate the protein molecules.

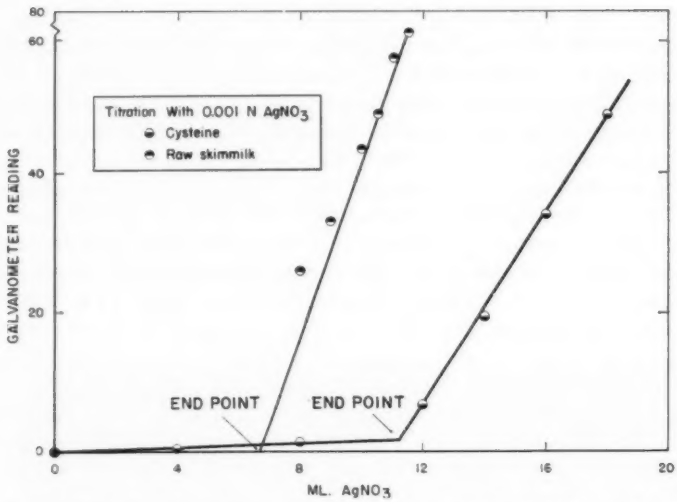


FIG. 2. -SH group titration curves for 1.75 mg. of cysteine.HCl and 25.0 ml. of raw skimmilk.

carried out under air or nitrogen. After the milk samples had reached the desired temperature, aliquots were removed immediately from the bath, cooled in ice water, and titrated with $AgNO_3$.

The above described procedure was modified as follows for the studies of the rate of the reaction. For the kinetic rate studies, 10.0-ml. samples of milk were heated in 25.0-ml. Erlenmeyer flasks immersed in the water bath at 79.0° C. The flasks then were removed from the bath at the desired intervals, and the contents were transferred quantitatively into a 250-ml. beaker containing the ice-cold sodium acetate solution. The remainder of the procedure has been described.

TABLE 1

Sulfhydryl groups in milk, whey, etc.

The values are calculated as milliequivalents of cysteine per liter of milk.

	-SH groups/l
Skimmilk	0.264
Raw whey	0.316
Boiled whey	0.022
Protein-free whey	0.000
Casein	0.000

[3] Applying a thin layer of mineral oil serves as an excellent method of reducing the evaporation of water, especially at the higher temperatures.

In order to minimize any protein denaturation other than that caused by the heat treatment, the milk samples were not agitated by stirring during the heat treatment.

TABLE 2

The effect of heat treatment on –SH groups in skimmilk

Temperature	m. eq. –SH/l.		Cooked flavor [a] score sample 2
	Sample 1	Sample 2	
(°C)			
untreated	0.264	0.308	—
28.5	.....	0.300	—
33.8	.....	0.328	—
38.0	0.296	0.320	—
43.5	.....	0.328	—
48.2	0.292	.....	
50.0	.....	0.292	—
58.5	0.284	0.280	—
69.0	0.224	0.272	+
78.2	0.180	.....	
80.0	.....	0.196	+
100.0 (one hr.)	0.072	0.092	+

(All values are the average of four separate titrations.)

[a] — = No "cooked" flavor, + = Strong "cooked" flavor.

RESULTS AND DISCUSSION

Sulfhydryl groups in milk and milk products. Although we have found that the quantity of -SH groups in milk and its derivatives varies from batch to batch of milk, even when obtained from the same farm, the values given in Table 1 show a typical distribution in milk, whey, casein, etc.

It appears, in agreement with Hutton (5) and Larson (8), that all the -SH groups of milk are in the whey proteins.

Effect of heating on -SH groups. The results of heating raw skimmilk in air on the -SH groups are presented in Table 2 and Figure 3. The values on unheated

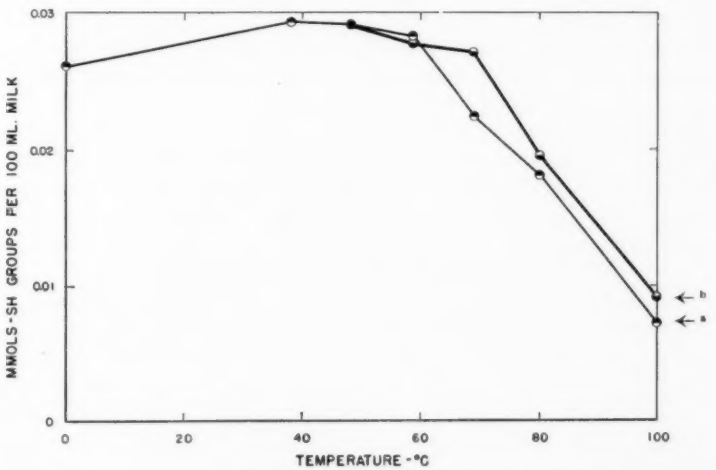


FIG. 3. The effect of temperature on –SH groups in two samples of skimmilk.

[a] Sample 1

[b] Sample 2

milk are numerically similar to those of Larson (8), who used an iodometric method of analysis. In contrast, Hutton (5), employing a procedure similar to that used in these studies, found only approximately one-half the quantity of -SH groups. It appears that the substitution of sodium acetate for NH_4NO_3 used by others (2, 5, 6) results in titration of the same number of -SH groups as the more tedious iodometric procedure. The use of NH_4NO_3 by Benesch (2) also may explain his inability to detect -SH groups in ovalbumin unless alcohol was added to the titration mixture. Studies are under way in our laboratory on the sulfhydryl groups of natural and denatured ovalbumin.

The values in Table 2 and Figure 3 suggest that heating causes a slight initial increase in titrable -SH groups and then with increased heating, a decrease.

It is seen in Figure 3 that there is only a slight change in -SH concentration to a certain temperature. Above this temperature, the -SH concentration decreases very rapidly with increasing temperature. The break in the curve at which this rapid change occurs is called the "critical temperature" and, in our experience, lies between 58 and 69° C. The variability of the "critical temperature" from batch to batch of milk may be related to the commercial experience that different samples of milk must be heated to different temperatures with variable holding times to achieve similar end-products.

Table 2 also shows approximate cooked flavor scores. These were evaluated organoleptically and are consequently largely subjective. However, it is seen that the appearance of cooked flavor was observed at the "critical temperature." This observation has been repeated many times.

The sulfhydryl content of nonfat dry milk. A number of samples of nonfat dry milk prepared by D. E. Mook were reconstituted and assayed for -SH groups. Some of the results are shown in Table 3 and Figure 4.

The two samples illustrated have "critical temperatures" at 71-77° C. It appears that the time of heating (holding-time) prior to drying at temperatures above that of the "critical temperature," has a pronounced effect on the loss of -SH groups, e.g., at 76.6° C. and holding-time of 5 minutes, the spray-dried milk contained 0.220 milliequivalents per 100 g. powder; when the holding time was lengthened to 20 minutes, the value fell to 0.163 milliequivalents per 100 g. The results indicate that the loss of -SH groups in whey proteins is a function of

TABLE 3

The effect of pre-heat treatment on –SH groups in nonfat dry milk

Temperature	Holding time	m. eq. –SH Groups/100 g.	
		roll-dried	spray-dried
(°C)	*(min.)*		
untreated		0.219	0.240
63.0	30	.....	0.243
71.4	5	0.186	0.243
71.4	10	.....	0.240
76.6	5	0.170	0.220
76.6	20	0.160	0.163
82.2	10	0.125	0.156
93.4	10	0.103	0.143

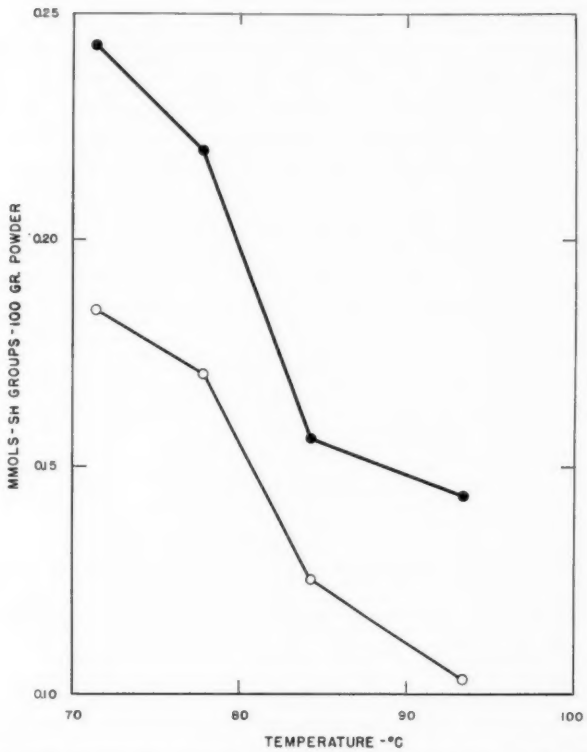


FIG. 4. The effect of pre-heat treatment on –SH groups in two samples of skimmilk powder.
O roll-dried powder
● spray-dried powder

time and temperature when the latter is above the "critical temperature."

The effect of flash-heat treatment on the -SH groups in reconstituted nonfat dry milk is given in Table 4. The loss of -SH groups at the higher temperatures (120-150° C.) is quite variable. This is a reflection of the fact that very slight variations in the "holding-time" in this type of heat treatment may result in marked differences in the physical properties of final product.

Kinetic studies. Larson and Jenness (10) reported that the loss of -SH groups in β-lactoglobulin by heat is a first order reaction. The results given in Table 5 confirm their findings on skimmed milk. The data presented in Table 5, which are typical of many experiments, show that a straight line is obtained when the log of the concentration of -SH groups is plotted against time.

The results indicate also that the methods used by Larson (8) and by us, the one based on o-iodosobenzoate and the other on the formation of silver mercaptide, are essentially equivalent.

The effect of oxidizing agents. The widespread use of oxidizing agents for

TABLE 4

The effect of flash-heat treatment on –SH groups in nonfat dry milk

Temperature (°C)	m. eq. –SH groups/100 g.
none	0.252
60.0	0.235
65.6	0.242
71.1	0.246
76.7	0.254
82.2	0.232
87.8	0.236
93.3	0.232
98.9	0.225
104.4	0.201
110.0	0.208
115.6	0.190
121.1	0.164
126.7	0.135
132.2	0.155
137.8	0.171
143.3	0.124
148.9	0.134

TABLE 5

Kinetic study of reaction involving loss of sulfhydryl groups at 79.0° C.

Time	m. eq. –SH groups/l.
0 min.	0.208
25.0 min.	0.182
50.0 min.	0.146
90.0 min.	0.110
20.0 hr.	0.020

TABLE 6

The effect of oxidizing agents on –SH groups in skimmilk at 25° C.

Sample	m. eq. –SH/l.
Raw skimmilk	0.206
Raw skimmilk + 0.03% H_2O_2	0.214
Raw skimmilk + 0.03% H_2O_2 + 8.2×10^{-4}% Fe as $FeCl_3$	0.190
Raw skimmilk + 0.03% H_2O_2 + 8.2×10^{-4}% Fe as $FeSO_4$	0.190
Raw skimmilk + 0.03% H_2O_2 + 8.2×10^{-5} Cu as $CuSO_4$	0.218
Raw skimmilk + 4.2×10^{-3}% $KBrO_3$	0.220

the "aging" of flour, which is usually associated with the oxidization of -SH groups, prompted us to apply this procedure to milk.

Unheated skimmed milk was treated with hydrogen peroxide or $KBrO_3$ with or without presence of small amounts of cupric, ferric, or ferrous salts (Table 6). The milk samples were analyzed for sulfhydryl groups 20 hours after addition of the oxidizing agents. The results in Table 6 show that under the conditions used, these oxidants had no effect on the -SH groups in milk.

Although $KBrO_3$ did not have any apparent oxidizing effect on the -SH groups in milk at room temperature, it was thought that small quantities of $KBrO_3$ may be able to lower the "critical temperature." The results of one such experiment are summarized in Table 7. It will be seen that within the experi-

TABLE 7

The effect of heat treatment on –SH groups in skimmilk, potassium bromate added

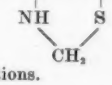
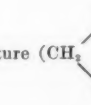
Temperature	m. eq. –SH/l.	
	Raw skimmilk	Raw skimmilk + 4.2×10^{-3}% $KBrO_3$
(°C.)		
38.0	0.26	0.28
48.0	0.24	0.23
59.0	lost	0.22
67.0	0.18	0.20
77.0	0.16	0.16

mental error of the method employed, $KBrO_3$ did not decrease the number of -SH groups at the various temperatures tested.

These findings may be explained on the basis that either the oxidizing agents were "used up" by other components of the milk before the -SH groups could be oxidized or that conversion of -SH to -SS- takes place, which then is reversed by reducing substances in the milk.[4]

SUMMARY

The sulfhydryl groups in milk proteins have been determined by the amperometric-argentometric method of Kolthoff. All or almost all the -SH groups in milk are present in the whey proteins. Heating milk causes an initial small rise in titrable -SH groups, followed by a marked decrease in -SH. The temperature at which the first sharp decrease in -SH groups is seen has been called the "criti-

[4] The disappearance of –SH groups observed when milk is heated may not necessarily be the result of oxidation to –SS–. Other reactions such as the formation of a sulfide (lanthionine) with the loss of H_2S from 2 molecules of –SH; thiazolidine formation (); djenkolic acid type structure (), etc. may account for the observations.

cal temperature." There is an apparent relation between the "critical temperature" and the appearance of cooked flavor. The loss of -SH groups found on heating milk above its "critical temperature" is a first order reaction. Heating in the presence or absence of dissolved oxygen or in the presence of oxidizing agents does not affect the value of the "critical temperature."

The method of Kolthoff is recommended as a useful tool for the evaluation of the effects of heat treatment on milk and milk products.

ACKNOWLEDGMENT

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THE EFFECT OF HEAT ON THE ALBUMIN AND GLOBULIN IN MILK

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Statements in the literature are conflicting as to whether the albumin and globulin in milk are coagulated by heat. Babcock (1) found no evidence of coagulation of albumin in heated milk in experiments in which he employed centrifugal separation. Rupp (4) obtained data that have been widely quoted as showing that no albumin is coagulated by heating milk at 62.8° C. (145° F.) for 30 minutes, that 5.75 per cent is coagulated by heating at 65.6° C. (150° F.) for 30 minutes, 12.75 per cent by heating at 68.3° C. (155° F.) for 30 minutes, and 30.87 per cent by heating at 71.1° C. (160° F.) for 30 minutes. Shahani and Sommer (5) reported that they observed about 9 per cent coagulation of albumin and 5 per cent coagulation of globulin in milk pasteurized by heating at 61.7° C. (143° F.) for 30 minutes. Orla-Jensen and Plattner (2) gave data on the coagulation of albumin in milk by heat, but made the statement that "l'albumine rendue insoluble par la chaleur doit etre precipitée avec la caséine."

Rowland (3) interpreted the situation in accordance with his data in a paper which apparently has not received the attention it deserves. He stated, "the term denaturation and not coagulation is applied to the change which takes place in the albumin and globulin when milk is heated. There is no visible coagulation, but the conditions determining the dispersion of the albumin and globulin are changed. The larger associated particles of denatured albumin and globulin are readily precipitated by acid and by salt solutions. The denaturation which takes place on heating milk is made evident, and its extent measured, by the precipitation of albumin and globulin in addition to casein when the heated milk is treated with those reagents which precipitate casein alone from heated milk." He obtained data and plotted curves for the percentages of albumin and globulin denatured by heating at temperatures in the range 65.2°–75.0° C. (150°–167° F.) for various times and determined the temperature coefficient to be 1.5 per degree Centigrade. A careful reading of the papers of Rupp and of Orla-Jensen and Plattner indicates that what they called coagulation was denaturation and that they obtained coagulation only by addition of acid or salt subsequent to heating.

We have attempted to obtain positive evidence of coagulation of the albumin and globulin of milk by heat by means of experiments in which the casein complex and other aggregates of similar size are removed from milk by means of the ultracentrifuge.

EXPERIMENTAL RESULTS AND DISCUSSION

A batch of fresh separator-skimmed milk was divided into two portions, one of which was heated in a closed container at 100° C. for 10 minutes. Samples of both the unheated and heated portions were spun for various lengths of time

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in an ultracentrifuge in order to separate different proportions of the casein complex and any other substances of similar or greater degrees of aggregation. The supernatant liquids were analyzed for total nitrogen and for nitrogen coagulable by acid, the latter being properly called casein nitrogen when determined on unheated milk. Values for the nitrogen coagulable by acid were plotted against the total nitrogen for both the unheated and heated milks. These plots are shown in Figure 1.

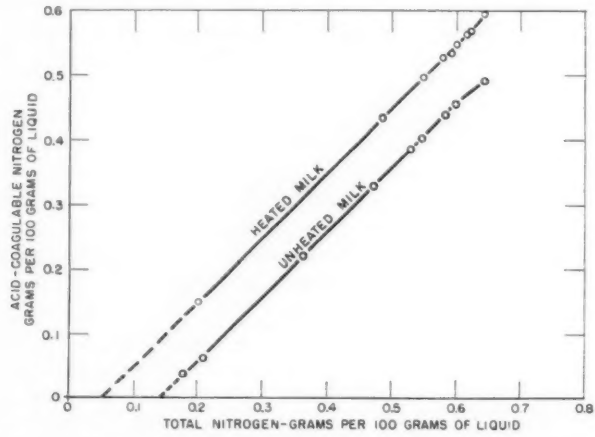

FIG. 1. The effect of heating on the coagulability of the non-casein proteins of milk.

It will be seen that the lines referring to the unheated and heated milks are practically straight and are parallel. Obviously, more nitrogenous material was precipitated by acid in the heated milks than in the unheated milks. The quantity of this acid-precipitated material was constant, regardless of the amount of casein removed by the ultracentrifuge, as shown by the constant difference between the lines. Hence, none of it was thrown out, even in the longer periods of spinning in the ultracentrifuge. The quantity of nitrogen in the nitrogenous material precipitated by acid after heating can be read from the figure as the distance between the plotted lines either on a vertical line or a horizontal line, that is, at constant total nitrogen or at constant acid-coagulable nitrogen. That distance corresponds to 0.090 ± 0.002 per cent nitrogen, which is equivalent to 0.57 per cent protein. This is practically the percentage of albumin plus globulin usually considered to be present in skimmilk. The magnitude of the values for acid-coagulable, non-casein, protein nitrogen thus indicate albumin and globulin, and the consistency of these values indicates that no albumin or globulin was thrown out by the ultracentrifuge under conditions adequate for throwing out the casein complex. It indicates also that these whey proteins were not coagulated. The heating, therefore, rendered the albumin and globulin susceptible to coagulation by the acid reagent employed for coagulating casein in raw milk. In other words, heating the skimmilk at 100° C. for 10 minutes

denatured its albumin and globulin but did not coagulate them nor aggregate them to a degree equal to that of the casein complex. Rennet or salts may be substituted for acid as the coagulating agent.

CONCLUSION

The albumin and globulin of milk may be denatured by heating the milk and are thus rendered coagulable by acid, salts, or rennet, but they are not coagulated or even aggregated to the size of the particles of the casein complex merely by heating.

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A CORRECTION

In the December, 1952, issue of the Journal in the article "Studies of Heated Milk. IV. Observations on Browning," by Stuart Patton, an error occurred on page 1061 in the legend for Figure 6. This should read ". . . . Lactose + casein (X —— X), lactose + enzymatically hydrolyzed casein (● —— ●) and lactose + acid hydrolyzed casein (■ —— ■)."

PEOPLE and EVENTS
in the Dairy Science World

A Tribute to Professor Mortensen

MARTIN MORTENSEN, Professor Emeritus and former head of Dairy Industry at Iowa State College, Ames, died March 13 in Houston, Texas, at the home of a daughter with whom he was visiting. Professor Mortensen was born in Denmark in 1872 and came to the United States at the age of twenty-one. After managing an Iowa creamery for several years, he entered Iowa State College in 1897 and took the course in dairy industry. After eight years in commercial work, he returned to the College in 1909 to become head of the Department of Dairy Industry, a position he held until 1938, when he reached the compulsory retirement age. As professor and emeritus professor, he continued his teaching and writing up to just a few months before his death.

Many honors came to Professor Mortensen for his leadership in the dairy industry. He was an honorary member of the American Dairy Science Association, which he had served as secretary-treasurer, vice-president, and president. He was a member of the Royal Agricultural Society of Denmark. He had visited Denmark and other European countries on several occasions as a member of the U. S. delegation to the World Dairy Congress. In 1927 he was knighted by the King of Denmark to the order of Ridder of Dannebrog; in 1950, he received the Commander's Cross of the Order of Dannebrog. In 1934, Kansas State College conferred an honorary LL.D. upon Professor Mortensen. In 1949, he received the faculty citation at Iowa State College for his years of service to that institution.

Professor Mortensen recently completed the manuscript for a history of dairying in Iowa. The hope is that this history may be published as a memorial volume through contributions made to the Alumni Achievement Fund of Iowa State College for this purpose.

Student Judging Contest to be Held in Boston

According to PROFESSOR G. M. TROUT, chairman of the committee on dairy products judging of the A.D.S.A., the 32nd annual collegiate students' international contest in the judging of dairy products will be held at Boston in October, 1953, in conjunction with the annual meetings of the Milk Industry Foundation and the International Association of Ice Cream Manufacturers. Dr. Trout reports that over 60 scholarships have been granted to winners of past contests. The influence of these men in industry, teaching, and research is widespread. The outstanding success of these past winners should be an inspiration to every dairy products team now in training for the 1953 contest. Here is Professor Trout's simple formula for being a contest winner: (a) know dairy products, (b) know the rules, and (c) use good judgment.

Should the Dating of Milk be Required?

One of the current problems in the milk industry has to do with the dating of milk. A number of plant operators believe that city health requirements which specify that bottled milk cannot be sold after a certain date stamped or printed on the container or bottle cap are no longer necessary in view of the generally higher quality of milk sold today as compared to the times when most of such ordinances were passed. Where such requirements still exist, there is a certain percentage of the milk sold in stores that must be returned at a loss—which means an added cost to the consumer. With an increasing percentage of milk being sold in stores, this problem grows in proportion.

Some health officers, on the other hand, contend that the dating requirement is necessary to protect the health of the consumer. Arguments supporting this point of view were presented in an article by GEORGE F. KIRCHOFF, Director of Food and Dairy Inspection of the Jefferson County Department of Health, Birmingham, Alabama, "Dating an Essential and Necessary Public Health Control Measure," published in the March, 1953, issue of the Milk Dealer. The opposite point of view was discussed in an article written by MURRAY TEIGH BLOOM and published in the March, 1953, issue of Redbook magazine. Mr. Bloom puts up a strong case against the dating requirement.

Dating was one of the topics discussed at the University of Illinois Milk Packaging Conference held April 8. Those participating in the panel discussion included representatives of public health, industry, and the legal profession. HARRY POLIKOFF, Counsellor-at-Law of New York City, discussed factors involved in the dating of milk.

Where the dating of milk is not required, it is necessary that plant operators pay particular attention to the procurement of a high-quality milk supply. It is also important that contamination of the milk subsequent to pasteurization be prevented, for it is in this manner that psychrophilic organisms are most likely to get into the finished package of milk.

American Dry Milk Institute Meeting

The American Dry Milk Institute held its 28th annual meeting in Chicago, April 21-22, 1953. A feature of the meeting was the "Technical Dinner" at which the following papers were presented.

1. The effect of heat treatment on the value of nonfat milk solids for bread making. ROBERT JENNESS, Department of Biochemistry, University of Minnesota.

2. Modification of the lactometer for determining the solids in milk. PAUL D. WATSON, Bureau of Dairy Industry, U.S.D.A., Washington.

3. Paying producers for fat and nonfat solids in milk. R. K. FROKER, Dean and Director, College of Agriculture, University of Wisconsin.

Klenzade Educational Seminar Held at Michigan State College

The 17th annual Klenzade Educational Seminar was held at the Kellogg Center, Michigan State College, East Lansing, March 26-28. A number of dairy scientists were among the 578 registered for the meeting, and several of them participated in the program. Panel discussions were held on institutional sanitation, mechanical dishwashing, canning plant sanitation, egg and poultry plant sanitation, high temperature equipment cleaning, can washing, corrosion of food equipment, corrosion of food utensils, circulation or in-place cleaning, dairy farm sanitation, sanitation chemistry, and sanitation bacteriology.

The address of welcome was given by PROFESSOR EARL WEAVER, Head of the Department of Dairy Husbandry, Michigan State College. C. B. SHOGREN, Vice-President of the Klenzade Company, in the final session presented the following six major problems confronting sanitarians today.

1. Lack of understanding of the principles of sanitation by all our people and a general lack of the teaching of these principles in our public schools.
2. The need for a greater appreciation on the part of industry of its responsibilities in public sanitation. The industry must do more and more "self-policing."
3. A lack of training in sanitation of the workers concerned in the growing, processing, and distribution of foods.
4. Lack of adequate funds and personnel for proper enforcement of existing sanitary regulations.
5. Conflicting and confusing rules and regulations of enforcement agencies and groups.
6. The limitations of man himself. A better appreciation of each individual's responsibility to society is needed by all members of that society. In this manner there will be better teamwork among people and a subordination of the individual's own selfish interests to those of the group as a whole.

Iowa Holds Dairy Industry Meeting

Approximately 500 people attended Dairy Industry Day at Iowa State College on March 24. Among the out-of-state speakers were: W. H. THOMPSON of the Bank for Cooperatives, Omaha, Nebr.; DR. H. C. OLSON of Oklahoma A. & M. College; EDWARD LAWTON of Dixon Ice Cream Co., Dixon, Ill.; GLENN BLAKE of Roszell Ice Cream Co., Peoria, Ill.; GEORGE ANDERSON of King Ventilating Co., Owatonna, Minn.; OTIS ROSS of National Pectin Co., Chicago; GEORGE GUNDLACH of G. P. Gundlach Co., Cincinnati; F. O. ROBITSCHECK of Colgate Palmolive Peet Co., New York; and H. E. EAGAN and MILTON HELD of the U. S. Public Health Service, Kansas City, Mo.

Total Solids as a Basis of Payment for Milk

The belief on the part of some people that milk should be paid for on a basis of its total solids introduces several important problems. First of all, a fast accurate method for measuring total solids must be made available. Scientists at the U.S.D.A., working under DR. GEORGE HOLM, are studying this problem at the present time. Another problem has to do with making the proper evaluation of the nonfat solids. If these solids are required to carry a higher percentage of the cost of milk, then the price of nonfat dried milk will have to rise accordingly. This will be no particular burden on the manufacturers of imitation dairy products such as filled milk, margarine, frozen desserts, and topping, but the bakers and food processors who are the principal users of nonfat dried milk at the present time may object. If the price of these solids gets too high, then bakers, for example, probably will reduce appreciably the amount of milk solids they use in their dough. Thus a readjustment in the market price of nonfat milk solids will be necessary to prevent a surplus from occurring unless the market for these solids in imitation dairy products expands to the point where it can absorb this surplus.

Prominent Dairyman Dies

JOHN BRANDT, for many years president of the Land O'Lakes Creameries, Inc., Minneapolis, Minn., died on March 5. Under his supervision, the Land O'Lakes cooperative has grown to be one of the largest in the country.

Report on Sanitary Milk Control

A research study on sanitary milk control sponsored and supervised by the committee on milk production, distribution, and quality, of the Agricultural Board and the Food and Nutrition Board of the National Research Council was published recently. The 174-page report, listed as Publication 250 of the National Academy of Sciences, National Research Council, Washington, D. C., was prepared by A. C. DAHLBERG, H. S. ADAMS, and M. E. HELD.

The report includes a study of the sanitary milk control and quality of eight selected cities. It is concluded that there is need for only a limited number of basic requirements to insure a wholesome milk supply. Some practices required by law are not essential and for that reason should be recommended rather than required. The report lists the significant factors in milk production and inspection of farms related to the sanitary quality of milk to be as follows: (1) prevention of contamination with pathogenic bacteria, (2) cleanliness in milk production methods, (3) clean milk, (4) milk cooling, (5) milk flavor control, (6) farm inspection, (7) number of milk samples tested per period per producer, and (8) milk production per cow.

Founder of Kraft Cheese Company Dies

JAMES L. KRAFT, founder of the Kraft Cheese Company, passed away, February 16, at the age of 78. Mr. Kraft was a man of many accomplishments. His greatest contribution to the dairy industry probably was his development of processed cheese. He started the Kraft Cheese Company in Chicago in 1903 by selling cheese directly to retail merchants. In 1921, he brought out the famous 5-pound loaf of processed cheese, which was followed by other types of packaged processed cheese.

Mr. Kraft was an active churchman and on December 31, 1952, was presented the second annual Gutenberg Award by the Chicago Bible Society for his outstanding service to all Christian causes. To further the careers of young men interested in agriculture, he actively supported the 4-H Club program. Mr. Kraft held honorary doctorates from Baylor University, the University of Redlands, Huntington College, and Linfield College.

Whither Dairy Processing Courses—Principles or Details?

A guest editorial

Dairy foods are the end products resulting from the application of scientific principles in dairy processing. In other words the foods, or products, are the results of science and are not science in themselves. Is it not logical, then, for teachers of dairy technology to emphasize scientific processing principles rather than end products?

When dairy processing was an art and the products were few, naturally the emphasis was upon the product. Today, even though science has largely replaced art in our processes and the number of products has increased, most of dairy technology instruction has not changed emphasis; the curricula still list the courses on a commodity basis — market milk, ice cream, butter, cheese, and concentrated milks. Such a viewpoint ignores the fact that many principles are common to all; it results in considerable duplication; and it does not provide for new or different dairy foods in the instructional scheme.

E. L. Jack

A much more logical approach to dairy technology instruction is to build around the scientific principles involved in the different dairy processes and then relate these processes to all dairy foods—new and old. New commodities can be included easily in the proper place, and unnecessary duplication can be avoided. Satisfactory results in this direction have been obtained at the University of California, where, in place of the usual commodity courses, a five-unit year course in the principles of dairy processing has been offered successfully for the past four years.

An eminent teacher of our time, DR. JOEL HILDEBRAND, Emeritus Professor of Chemistry, University of California, declares: "The only courses that should be offered, in my opinion, are those dealing with basic principles and ideas." Dr. Hildebrand adds: "Fields . . . in which knowledge is least exact and systematized are the ones likely to offer the largest number of courses." Dairy technology has developed basic principles, and the knowledge has been systematized in research activities; the subject matter of the courses also should be arranged in orderly fashion. In analagous fields, such as chemical engineering and food technology, the subject matter is organized around processing principles rather than commodities—an arrangement made necessary by sheer numbers of commodities if for no other reason. If dairy technology is to hold its place at the forefront of the food-processing field, the instruction must deal primarily with the scientific principles of dairy-food processing and secondarily with the resulting products. The student must learn the principles in college; he can and will learn in industry the details applicable to the different foods.

E. L. JACK, *Chairman*
Department of Dairy Industry
University of California, Davis

ASSOCIATION ANNOUNCEMENTS

48TH ANNUAL MEETING

UNIVERSITY OF WISCONSIN

MADISON

JUNE 22-24, 1953

Registration and Housing

Registration and housing assignment headquarters will be in Babcock Hall from 2 P.M. to 10 P.M., June 21, and 8 A.M. to 5 P.M., June 22-24. Housing facilities will be available in University dormitories, residence halls, hotels, and motels. Except for hotels, all housing assignments will be made by the Housing Committee. Meals will be available, beginning Monday, in the dormitory cafeterias, and Wisconsin Union dining hall, by advance reservation only.

Announcements of the meeting, a card for advanced registration and room reservations, and other detailed information have been sent to individual members of the Association. All requests for lodging accommodations will be confirmed.

Committee and Special Meetings

Groups desiring rooms for committee or special meetings, and those making group reservations for special breakfasts, luncheons, or dinners should write to: Rooms Committee, A.D.S.A., Babcock Hall, University of Wisconsin, Madison 6, Wisconsin. Please state the time of meeting and the number of persons expected to participate. All special breakfast, luncheon, or dinner reservations must be in by June 1, 1953.

Facilities for Sessions

Equipment will be available in all lecture rooms for the projection of 3.25 $\times$ 4 in. and 2 $\times$ 2 in. slides. Those wishing other projection equipment should notify Facilities for Sessions Committee of A.D.S.A., Babcock Hall, University of Wisconsin.

Title, list of authors, date and number, if available, should be on all mimeographed and printed material which is to be distributed at meetings.

Employment Clearing Center

An employment clearing house will be in operation during the session. Information will be on file pertaining to prospective employees, primarily, college graduates holding B.S., M.S., or Ph.D. degrees. Employee application blanks will be made available at the Dairy Department offices of the various state colleges and universities prior to the meeting. These employment applications should be filled in and mailed in advance to W. H. Chilson, Department of Dairy and Food Industries, University of Wisconsin, Madison, Wisconsin.

Employers are invited to attend the meetings and to contact the employment desk to obtain information about individual applicants. Interviews will be arranged at the convenience of the employer and the prospective employee.

Employers may also file information folders on available positions for personnel trained in Dairy Science. Such folders might then be examined by individuals looking for employment and interviews would be arranged for.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEW

270. Refrigerating Data Book, Applications Volume (4th ed.). H. C. DIEHL, Editor-in-Chief. Published by American Society of Refrigerating Engineers. 1952.

The 1952 Applications Volume covers the use of the science and art of refrigeration and the application of refrigerating machinery and associated equipment to the preservation and transportation of foods and to the many industrial processes requiring refrigeration. The volume is made up of 8 sections containing 65 chapters. Each section editor is a recognized authority in the particular field of refrigeration application, and each chapter is contributed by a specialist. Chapters in the first 4 sections are of special interest to workers in dairy industry: (Section I) Theories and Methods of Quick Freezing; Fruits and Vegetables; Ice Cream; Poultry and Egg Products; Storage of Frozen Foods; and Frozen Fruit Juice Concentrates; (Section II, Refrigeration in Food Manufacture) Milk Plants; Butter Manufacture; and Cheese Manufacture; (Section III, Refrigerated Warehouse Practice) Refrigerated Warehouses; Commodity Storage Requirements; Candies, Nuts, Dried Fruits and Vegetables; Storage of Canned Foods; and Locker Plants; (Section IV, Refrigerated Food Distribution) Refrigerated Trucks and Trailers; Refrigerator Cars, Dry Ice in Food Distribution; and Commercial Refrigerators. The only chapter in succeeding sections of direct interest to the dairy industry worker is Ice Manufacture, in Section VI, Industrial Applications of Refrigeration. It is to be hoped that in a future edition the editors will either enlarge the chapter for Market Milk to cover the bulk cooling of milk on the farm or add a full chapter devoted to mechanically refrigerated milk cooling. The design aspects of refrigerating machinery are not found in this book but in the Refrigerating Data Book, Basic Volume, ASRE, 1951.

L. M. Dorsey

CHEESE

S. L. TUCKEY, SECTION EDITOR

271. Die Dynamik der Labg'erinnung in der Käserei (The dynamics of rennet action in cheese making). English summary. M. E. SCHULZ. Die Milchwissenschaft, **6**, 3: 74-82, 4: 112-118. March-April, 1951.

The author visualizes three spheres of rennet activity, namely: molecular dynamics, microdynamics, and macrodynamics. He believes that molecular dynamics involve the electric charge of the individual protein molecules, which possibly exhibit a bipole movement and thereby permit the formation of fibers or threads.

Concerning the moisture in the curd the author explains that the voids between the protein fibers or threads are filled with free or loose water, whereas the small channels or capillaries within the protein fibers or threads hold the capillary moisture, which has been estimated to range from 10 to 30% of the total moisture in the curd. The remaining 40% moisture in the curd is in the form of bound water, which cannot be removed by physical pressure but can be removed by appropriate treatment of the curd with alcohol.

Curd shrinkage is influenced by (a) curd shrinkage tension, (b) curd shrinkage pressure upon whey, and (c) resistance offered by the curd to the movement of whey.

Slow renneting of milk may be caused by (a) presence of enzyme inhibitors, (b) slow rennet action, (c) weak curd, and (d) retarded shrinkage of curd. I. Peters

272. Verwendung des untravioletten Lichtes zum Zwecke der Qualitatsverbesserung und Arbeitserleichterung in Käsereien (The use of ultraviolet light in cheese factories for the purpose of quality improvement and work simplification). English summary. G. GODBERSEN. Die Milchwissenchaft. **6**, 3: 82-85. March, 1951.

Camembert cheese ripened for 18 days with and without periodical exposure to ultraviolet light showed no organoleptically detectable difference at the end of the test period.

Daily treatments with a low vapor U.V. lamp located 500 mm. from the surface of the cheese retarded surface growth of all types of microorganisms, provided treatments were started at the beginning of the ripening period and were performed daily. Such cheese when wrapped in tin-foil developed luxurious growth of white

mold and ripened normally. Other cheese treated late in the ripening period or at less frequent intervals developed growth of yeasts, which were replaced by *Bacterium linens* subsequent to wrapping the cheese in tin foil.

The untreated cheese developed surface growth of yeasts and spots of blue-green mold; after such cheese was wrapped in tin foil, the mold continued to grow, whereas the yeasts were replaced by *B. linens*.

Results of a similar experiment with Tilsit cheese showed that treatment of such cheese with ultraviolet light during a period of 51 days did not result in a better quality ripened cheese.

I. Peters

273. Divider for cheese molds. M. F. Pullen. U. S. Patent 2,625,877. 3 claims. Jan. 20, 1953. Official Gaz. U. S. Pat. Office, **666**, 3: 722. 1953.

A rectangular cheese mold having sheet metal dividers for producing a number of loaf-shaped cheeses within the one mold. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

274. The determination of penicillin in milk. W. Gogas and A. K. Bicknell, Wayne Univ., Detroit. Milk Plant Monthly, **42**, 2: 26. Feb., 1953.

A simple method for the detection of penicillin is as follows: (1) 100 ml. of the seed agar is inoculated with 2.0 ml. of a 48-hr. broth culture of *B. substilis*, composed largely of spores. (2) Approximately 15 ml. of the seeded agar is placed in sterile petrie dishes and allowed to harden. The glass covers of the petrie dishes are replaced with either previously sterilized porcelain covers or sterile glass covers having filter paper as an inside lining. (3) Plates are inverted and incubated for 2½ hr. They then may be removed and stored in the refrigerator until needed. (4) When a penicillin determination is needed, a seeded agar plate is divided into 4 equal sections with a glass marking pencil. Sterile filter paper discs (S and S, No. 740-E, 12.7 mm. in dia.) are wet by capillary action with the milk to be tested and placed aseptically on the surface of the seeded agar. The amount of milk absorbed by the disc is approximately 0.023 ml. (5) Plates are incubated at 37° C. for 2 hr., or less. Within this time a zone of inhibition can be observed and measured. If desired, penicillin discs, varying in concentration from 0.1 unit per ml. to 10 units per ml. may be used as reference standards. (6) In order to determine whether the antibiotic present is penicillin, penicillinase discs can be impregnated with the unknown sample of milk and placed on the same petrie dish, as indicated in (4). If no zone of inhibition appears around the penicillinase disc, while there is one around the blank containing the unknown, penicillin is present. Other antibiotics can be tested similarly. C. J. Babcock

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

275. A suggested modified Babcock procedure for testing half-and-half. R. B. Maxcy, Geo. J. Meyer Mfg. Co., Cudahy, Wis. Milk Dealer, **42**, 4: 42, 43, 52-54. Jan., 1953.

A modified test was evolved in which the usual Babcock equipment and basic procedure were used for determining the fat content of homogenized half-and-half containing 8-13% milk fat. The major changes were the use of an ice cream test bottle, the addition of 9 ml. of trisodium citrate, and the addition of 2 ml. of n-propyl alcohol prior to the addition of the acid. The results were in close agreement with the results of the Mojonnier test. The detailed procedure for this method is: (1) Temper the milk, acid, trisodium citrate, and n-propyl alcohol to approximately 70° F. (2) Weigh a 9.0-g. sample of the half-and-half into an ice cream test bottle which is calibrated to test up to 20%. (3) Add 2.0 ml. of n-propyl alcohol, followed by approximately 9 ml. of trisodium citrate (a saturated solution at room temperature). (4) Add 17.5 ml. of sulfuric acid of 1.825-1.830 sp. gr. in 3 separate portions and after each addition shake the mixtures for 2 min. on a mechanical agitator. (5) Centrifuge and add hot water in accordance with the regular Babcock procedure for milk. (6) Temper the bottles in a water bath at 135-140° F. for 5 min., add glymol down the side of the neck, and read to the nearest 0.1% by means of a pair of sharp pointed dividers. C. J. Babcock

276. Centrifugal tester. A. H. Boileau (assignor to Cherry-Burrell Corp.). U. S. Patent 2,628,773. 3 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 678. 1953.

A Babcock fat test centrifuge, designed to provide uniform temperature throughout. A built-in duct leads from the top center in the vicinity of the shaft, around the side of the centrifuge and connects to an outlet on the bottom center. When the centrifuge is in operation, air circulates through this duct and maintains a uniform temperature. R. Whitaker

DAIRY ENGINEERING

C. W. HALL, SECTION EDITOR

277. Lift plug valve. F. Hinrichs (assignor to Tri-Clover Machine Co.). U. S. Patent 2,628,059. 2 claims. Feb. 10, 1953. Official Gaz. U. S. Pat. Office, **667**, 2: 410. 1953.

A sanitary plug type valve, which may be lifted slightly out of its seat when being turned. The lifting action is provided by having the handle attached to the valve proper by a pivot. This acts as a fulcrum when the handle is given a rocking motion. R. Whitaker

278. Receptacle filler. R. E. Bodendoerfer (assignor to Cherry-Burrell Corp.). U. S. Pat-

ent 2,628,759. 4 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 674. 1953.

Details are given for the construction of a valve for a milk or other liquid bottle filler.
R. Whitaker

279. Delayed-action pulsator valve. J. C. MARLOW. U. S. Patent 2,625,948. 2 claims. Jan. 20, 1953. Official Gaz. U. S. Pat. Office, **666**, 3: 742. 1953.

A device for controlling the rate of pulsations of a milking machine. R. Whitaker

280. Milk line nipple. M. J. SMITH, (assignor to Rite-Way Products Co.). U. S. Patent 2,626,590. 5 claims. Jan. 27, 1953. Official Gaz. U. S. Pat. Office, **666**, 4: 979. 1953.

A manifold or milking machine claw consisting of 4 outlets to which hoses leading to the teat cups are attached. R. Whitaker

281. Milk cooler. F. A. HANSEN. U. S. Patent 2,626,784. 2 claims. Jan. 27, 1953. Official Gaz. U. S. Pat. Office, **666**, 4: 1037. 1953.

A metal ring designed to rest on the top of the necked portion of a milk can for distributing cooling water in a uniform film to flow over the outside surface to cool the milk within the can.
R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

282. Milk vending goes strong at Sampson. Anon. Milk Dealer, **42**, 4: 40-41, 81. Jan., 1953.

An initial installation of 9 dual flavor vending machines proved so popular that volume soon necessitated installing 10 additional machines at Sampson Air Force Base, near Geneva, N. Y. J. Souhan and Sons in Seneca Falls, N. Y., supply milk to the vending machines in conical half-pint containers. Milk and chocolate milk are priced at 10 cents, and the machines are set to accept either 2 nickels or a dime. The September, 1952, vendor sales figures for this base shows that 454 soft drink coin coolers dispensed 820,000 bottles of cola, 29 fruit juice vendors sold 30,000 cans of juice, and 19 milk machines vended 80,000 half-pint containers of milk and chocolate milk. Thus, although numerically the dairy vendors amounted to only about 4% of all the beverage machines on location, they tallied some 15% of the total dollar gross.
C. J. Babcock

283. The year ahead. H. A. RUEHE, Univ. of Ill., Urbana. Milk Plant Monthly, **42**, 1: 28-31. Jan., 1953.

The dairy processing business is fundamentally like any other manufacturing business. It is composed of 4 phases: (1) purchase of raw materials, (2) manufacturing processes, (3) advertising and selling, and (4) financing. After a brief discussion of these phases of the dairy business, it is stated that: (1) Our supply of basic raw materials in the dairy field has not kept pace with our rapidly increased population. To compensate for this lack, better financing methods, better utilization of all the nutrients of milk, better processing and marketing, and more efficient financing will be needed. (2) To maintain its share of the consumer food dollar, even at the present level, as well as to equalize the cost carried by the fat portion and the milk solids-not-fat portion of milk, the dairy industry must inaugurate a realistic merchandising program which will include not only advertising and selling but also education in nutrition.
C. J. Babcock

284. Dairy foods win favor with flavor. H. A. BENDIXEN, State Coll. of Washington, Pullman. Am. Dairy Prod. Rev., **15**, 1: 10, 11, 16-21. Jan., 1953.

Trends and problems of the dairy industry are reviewed. The importance of flavor and quality in dairy products is stressed, and factors affecting these characteristics are discussed. In butter, in which flavor is an important sales factor, the author believes that the use of official grades would be of benefit to the industry.
T. J. Claydon

285. Labor comparisons of take-down and in-place cleaning of dairy plant pipe lines. GLENN T. NELSON, Ore. State College, Corvallis. Milk Plant Monthly, **42**, 2: 52-54. Feb., 1953.

To clean 1,050 ft. of pipe line in a milk plant, take-down cleaning required an average of 12.5 man-hours per day, compared to 2.9 man-hours for in-place cleaning. With take-down cleaning, assembling and disassembling required 6.2 man-hours, or approximately half of the cleaning time. Washing and rinsing took 4.1 man-hours. Carrying the pipe to the wash tank, removing gaskets, replacing gaskets, and returning the pipe to the assembly point accounted for 2.2 man-hours per day. The labor-saving time using in-place cleaning instead of take-down cleaning in this plant, therefore, was 9.6 man-hours per day. Other observations from the study indicate: (1) Wash racks on wheels make breakdown cleaning easier. (2) Addition of the fourth man to the cleaning crew resulted in some waiting at the wash rack. (3) The height of the pipelines made it necessary for some workers to use boxes or stools in assembling and disassembling, which increased the time of the operation. (4) Cleaning the pipe lines in-place makes it possible to mount them higher. There is thus less chance to damage the pipes and fittings, and this permits use of less expensive piping. Finally, (5) members of cleaning crews become less fatigued in operating the circulation cleaning system than when disassembling and cleaning the pipes and fittings. As a result, the cleap-up crew maintained greater efficiency. C. J. Babcock

286. Plant development and employee benefits. Anon. Ice Cream Rev., **36**, 7: 46, 47, 83, 84. Feb., 1953.

The article describes a profit sharing and retirement program which was put into effect in 1945 by the Hygeia Milk Products Co., Harlingen, Texas. Under the profit sharing feature, the company contributes to the fund on Dec. 1 of each year a sum based on the profits of the preceding 11 mo. Each employee who has been with the company for 1 yr. is credited with a proportionate share of this fund, based on his salary in relation to the salaries of all participants.

One-fourth of the sum credited to an employee may be withdrawn in cash, and the balance remains as a credit. The money in the fund is administered by a board of three participants who have authority to invest the money and make loans to employees. If an employee leaves the company, he is entitled to a 10% withdrawal of the money which has been credited to his account for each full year of employment. If sickness or disability is the reason for leaving the company, the full amount of money is paid. In case of death, payment due is made to the employee's beneficiaries.

The retirement program provides a pension plan for all employees who remain with the company until the age of 65 yr. Proportionate benefits are provided for employees who do not remain in employment until the retirement age. Participation in this program is available to any employee after 1 yr. of service. The employee contributes 2% of his salary, and the company pays the remainder. Retirement income provided under the plan amounts to 1% of the employee's salary for each year he participates in the plan. The maximum salary upon which retirement is figured is $350 per month, and the maximum monthly retirement income amounts to 35% of the monthly salary.

W. J. Caulfield

287. Survey reveals milk drinking habits of California consumers. W. B. Woodburn, Calif. Dairy Ind. Advisory Board, Sacramento. Milk Dealer, **42**, 4: 86-89. Jan., 1953.

A survey of consumer milk-drinking habits of 513 representative families in Los Angeles and 424 in Oakland showed that 75% of the fluid milk used during the week studied was consumed as a beverage. Almost 90% of the families used milk in food preparation. From 7 to 15% of the whole milk purchased was utilized in that way, the largest proportion in baking. About 2/3 of the families used milk on cereals, and 1/3 in tea and coffee. About 45% of the half-and-half was used in tea and coffee, 37% in cereals, 7% in food preparation. Only 7% of the families bought table cream. Evaporated milk was purchased by 45% of the families in Oakland and 37% of those in Los Angeles. About 1/3 of this milk was used in tea and coffee; about ¼ in infant feeding; 7% on cereals; 6-9% as a beverage; 20% in food preparation; and 7-12% for pet food. Only about 3% of the families used either nonfat or whole dry milk. From 60-68% of the families used cottage cheese.

Eight products, namely, plain whole milk, homogenized milk, multi-vitamin milk, half-and-half, evaporated milk, cottage cheese, American Cheddar cheese, and ice cream accounted for 90.7% of the total milk equivalent quarts, during the week studied, in Oakland and 87.1% in Los Angeles.

C. J. Babcock

288. What is the dairy industry's responsibility to our universities? H. F. Judkins, Vice-President, Natl. Dairy Products Co., Inc., New York. Ice Cream Rev., **36**, 7: 52, 55, 56-60, 62. Feb., 1953.

The author suggests that if industry wishes to use the services offered by the universities it should: (1) Tell the university of the problems on which it desires help. (2) Put the results of university research to work. (3) Get behind state appropriation requests for the work of the university when necessary. (4) Explain to the university the opportunities in the dairy industry and the type of man power needed. (5) To so handle and train the university graduate as to enable him to live the most happy and useful life of which he is capable. Of these 5 items, it is in the area of the two last that industry should direct its immediate attention.

Opportunities for key positions in 14 specific fields in the dairy industry are cited as follows: (1) procurement of raw material, (2) purchasing of equipment and supplies, (3) plant operations, (4) research and quality control, (5) engineering, (6) transportation, (7) accounting, (8) insurance, (9) taxes, (10) legal, (11) personnel, (12) public relations, (13) sales, and (14) executive management. A number of specific qualifications are presented for handling these key positions.

Two programs of training in preparation for employment in the dairy industry field are suggested. In the first plan, superior students from the school of business administration would take courses in dairy manufacturing to determine whether they might like to use their business training in the dairy field. In the second plan, students majoring in dairy industry who show a strong leaning toward the business field should be encouraged to take several undergraduate courses in the field of business administration. A 6-yr. college training program represents the ideal, in the author's opinion, for future leaders in the dairy industry.

W. J. Caulfield

289. Improve your business management in 1953. Fred Merish. Milk Plant Monthly, **42**, 1: 48-51. Jan., 1953.

To prepare for the deflationary period which some economists predict will start in 1953, milk plant operators should improve their sales con-

trol and cost control. It may be harder to earn a satisfactory net in 1953 than it was in 1952. A carefully planned program is necessary. Operations should be planned by means of a quarterly budget. Because of the vast change of pace today, it is unwise to budget for a longer period. In planning, the operator should remember that he can cut his variable expenses when volume drops to a lower bracket. He cannot control a constant or fixed expense. He should, therefore, avoid increasing fixed expenses and try to reduce indebtedness.

C. J. Babcock

290. Watch your working capital. Fred Merish. Milk Plant Monthly, **42**, 2: 56-60. Feb., 1953.

Money is getting "tight." It is harder to borrow, and interest rates are going up. It is important for the milk plant operator to learn how to operate most successfully when money is tight. To meet this situation it is necessary for the operator to know how to apply certain yardsticks to his financial operations. This is possible by the use of ratios. (1) A ratio of at least 2.5 to 1.0 of current assets to current liabilities should be maintained. Current liabilities deducted from current assets equals working capital. The desirable working capital varies with operating conditions. (2) It is suggested that a ratio of 8 or 10 to 1.0 of sales to working capital be maintained. (3) A ratio of 1.5 to 1.0 of fixed assets to working capital is considered adequate. As the plant ages, this ratio will increase unless replacements are kept at top level. C. J. Babcock

291. Bulk tank system of handling farm milk. A. C. Fisher. General Ice Cream Corp., Schenectady, N. Y. Milk Dealer, **42**, 4: 45, 46, 58-62. Jan., 1953.

The handling of farm milk in bulk through the medium of refrigerated tanks has passed the experimental stage and is now in a phase of adoption and adaptation. This method of handling milk is discussed from the standpoint of quality, cost, the small producer, and tank size. At the present time, the average investment for a tank of 200-gal. capacity is about $2,300, completely equipped and installed. On a 10-can or 100-gallon tank, this cost would be approximately $1,500-$1,600. Among the problems still to be solved is setting up routes, as the installation of bulk handling must be done on an individual route basis. One tank will take the place of 2 can routes, and the problem of what to do with the small producer on these routes must be solved. This type of milk handling increased the tendency of raiding of supply by another co-operative or competing dealer. This poses a serious threat to the stability of a supply area. Furthermore, this type of milk handling makes it possible for gypsy haulers to accumulate tank loads and dispose of them to the highest bidder. Perfection has not been reached in the present bulk handling operation. Four years of experience, however, indicates that it is sound economically and from a quality standpoint. C. J. Babcock

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

292. Wintering dairy heifers in Arkansas. L. Ratcliff, O. T. Stallcup, J. L. Cason, and E. D. Moore. Ark. Farm Research, **1**, 3: 6. Fall, 1952.

Alfalfa hay when fed to heifers 12-14 mo. of age produced gains of 1.37 lb. per day as compared to 0.41 lb. per day for heifers fed sericea lespedeza hay. Mixed alfalfa-orchard grass hay produced gains of 1.25 lb. per day as compared to 0.53 lb. per day for yearling heifers fed sericea lespedeza hay. Yearling heifers fed Korean lespedeza hay gained 1.03 lb. per day as compared to 0.46 lb. per day for animals fed sericea lespedeza hay. Heifers 6-7 mo. of age gained 0.88 lb. per day on a diet of Korean lespedeza hay plus ¾ lb. grain per 100 lb. body weight as compared to 0.74 lb. per day for similar animals fed hay plus ½ lb. of grain per 100 lb. body weight.

O. T. Stallcup

293. Milking machine. A. E. Mayer. U. S. Patent 2,627,840. 2 claims. Feb. 10, 1953. Official Gaz. U. S. Pat. Office, **667**, 2: 346. 1953.

A milking machine of the pulsator type having two milk receiving vessels of dissimilar size. Three of the 4 teat cups are connected to the larger vessel, and the 4th teat cup is connected to the smaller. Provision is made to have all 4 teat cups empty into the larger reservoir if desired, but when one quarter of the udder is diseased, the milk from it may be kept separate from the other milk. R. Whitaker

294. Electrical calf weaner. L. M. Johnston. U. S. Patent 2,628,592. 2 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 2: 626. 1953.

A receptacle holding a battery, a switch, and a shock-producing vibrator is attached to the head of a calf by a halter. When the calf attempts to suckle, its up-tilted head closes the switch and the electrical circuit is completed when an electrode touches the cow's udder. On closing the circuit, the vibrator operates and gives a mild shock to the nose of the calf.

R. Whitaker

295. Milk transfer unit for automatic milking machines. R. E. Reeve. U. S. Patent 2,628,589. 2 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 625. 1953.

Details are given for the construction of a device suspended beneath a cow by a strap passing over her back, to which the milker claw, air hose, and milk tube are attached.

R. Whitaker

296. Feeder pail valve assembly. J. M. COYNER. U. S. Patent 2,628,591. 3 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 626. 1953.

A pail designed for feeding calves. A feeding nipple is attached to a short piece of tubing fitted to a hole in the side of the pail in the bottom corner. A flap valve is mounted in the tubing at the point where the tubing is attached to the pail wall. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

297. Ice cream formulas for diabetics. B. E. HUBBELL, JR., Univ. of Calif., Davis. Ice Cream Rev., **36**, 7: 98, 99. Feb., 1953.

The composition suggested for a diabetic ice cream is as follows: 12-14% butterfat, 9% serum solids, 15% sorbitol, or a blend of equal parts of 70% d-sorbitol solution and commercial grade powdered mannitol, 0.01% saccharin or sodium sueryl, and a stabilizer emulsifier which is free of added sugar. It is suggested that low lactose nonfat dry milk solids, containing approximately 36% lactose, be substituted for nonfat dry milk solids in diabetic ice cream mix.

Packages of diabetic ice cream should be labeled to indicate the calories per unit weight, calorie value of protein, fat, and carbohydrate content, and name of sweetening agents used.
W. J. Caulfield

298. Mixing device for frozen confections. C. REED. U. S. Patent 2,626,132. 19 claims. Jan. 20, 1953. Official Gaz. U. S. Pat. Office, **666**, 3: 798. 1953.

A mixer for milk shakes and soft ice cream and ice milk, consisting of a conical shaped chamber in which there rotates a tapering auger type of agitator is described. The convolutions of the beater are closer together at the small bottom end than at the wider top end. The conical cup is raised and lowered by hand to engage the motor driven beater, which is held in a fixed position. The product is discharged from the bottom of the cone-shaped cup.
R. Whitaker

299. Apparatus for converting hard ice cream or frozen confections to a product of softer consistency. W. S. FREDENHAGEN and M. S. SCHMIDT. U. S. Patent 2,628,082. 4 claims. Feb. 10, 1953. Official Gaz. U. S. Patent Office, **667**, 2: 417. 1953.

Hard ice cream is softened by this device to the consistency of ice cream as it is drawn from the freezer. It consists of two double-pronged beaters, which rotate in the manner of an egg beater in a tapered bottomless sleeve slightly larger than the beaters. A paper cup, of such diameter that it slips over the bottom of the sleeve, constitutes the bottom of the mixing chambers and forms the container in which the ice cream is dispensed. R. Whitaker

300. Egg substitute admixture and process of producing the same. M. M. HEALY. U. S. Patent 2,628,904. 7 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 715. 1953.

A powdered egg substitute consisting of dry skimmilk, egg albumen, rice and tapioca starch, and a gas-forming agent. R. Whitaker

301. Ice cream packaging machine. W. H. ALLISON. U. S. Patent 2,628,756. 6 claims. Feb. 17, 1953. Official Gaz. U. S. Pat. Office, **667**, 3: 673. 1953.

A dipper for producing cylindrical-shaped portions of ice cream. R. Whitaker.

302. Apparatus for molding and packaging ice cream. K. F. SCHULZE. U. S. Patent 2,627,824. 4 claims. Feb. 10, 1953. Official Gaz. U. S. Pat. Office, **667**, 2: 341. 1953.

Ice cream from the freezer is filled into a paper-lined cylindrical mold made from wire.
R. Whitaker

303. Frozen confection package and container therefor. J. VALENTA. U. S. Patent 2,627,472. 7 claims. Feb. 3, 1953. Official Gaz. U. S. Pat. Office, **667**, 1: 165. 1953.

A package for a frozen stick novelty of rectangular shape. A slit provided in the bottom folds, allowing the package to be closed around the confection with the stick protruding. At time of consumption, the top portion of the package can be torn off and discarded. As the confection is eaten, the stick can be pushed upward to expose additional product.
R. Whitaker

304. Apparatus for manufacturing ice cream cake rolls and the like. P. M. ANGELL and W. J. BENN (assignors to Newly Weds Baking Co.). U. S. Patent 2,627,236. 4 claims. Feb. 3, 1953. Official Gaz. U. S. Pat. Office, **667**, 1: 99. 1953.

A layer of ice cream is spread on a sheet of cake and the two layers are rolled and properly hardened. The cake roll is sliced to form individual servings by this device, which consists of oscillating knife blades against which the roll is pressed. R. Whitaker

305. Apparatus for manufacturing ice cream cake confections and the like. P. M. ANGELL, (assignor to Newly Weds Baking Co.). U. S. Patent 2,627,237. 7 claims. Feb. 3, 1953. Official Gaz. U. S. Pat. Office, **667**, 1: 99. 1953.

An ice cream cake roll slicer, designed to accomplish the same results as described in Abstract No. 304, except in this case the knife blades are stationary and the cake roll is rotated as it is pressed against the blades.
R. Whitaker

306. Stick-holding device. D. GLAZER (assignor to Frigie Corporation of America). U. S. Patent 2,626,830. 2 claims. Jan. 27, 1953. Official Gaz. U. S. Pat. Office, **666**, 4: 1049. 1953.

Structural details are given for the design of a device for holding sticks in position in a brine tank frozen novelty mold. A mechanism, easily operated by pressure on a bar near the handle, releases the sticks when the mold is defrosted. R. Whitaker

307. Agitating apparatus for tray ice cream freezers. W. W. PARKER (assignor to W. W. Parker, Jr. and R. N. Parker). U. S. Patent 2,626,134. 2 claims. Jan. 20, 1953. Official Gaz. U. S. Pat. Office, **666**, 3: 798. 1953.

A hand operated U-shaped agitator, which fits into the tray of a household refrigerator, for agitating ice cream and other frozen products during freezing. R. Whitaker

MILK AND CREAM

E. O. HERREID, SECTION EDITOR

308. Process for the manufacture of stabilized milk products. R. E. MEADE (assignor to Western Condensing Co.). U. S. Patent 2,627,463. 7 claims. Feb. 3, 1953. Official Gaz. U. S. Pat. Office, **667**, 1: 164. 1953.

Liquid dairy products, such as whey, are concentrated and treated so that the lactose crystallizes as the alpha monohydrate, which then is spray dried and forms two fractions, one containing a high proportion of crystallized alpha lactose, and a second containing less crystallized lactose. The second fraction is returned to the drier and mixed with the raw feed to facilitate production of larger amounts of the first one. R. Whitaker

309. Regulation of the fat content of milk products. P. A. DAHLSTEDT (assignor to Aktiebolaget Separator). U. S. Patent 2,628,023. 2 claims. Feb. 10, 1953. Official Gaz. U. S. Patent Office, **667**, 2: 399. 1953.

The cream and skimmilk lines from an air tight type of separator are each equipped with a cell which continuously measures the electrical resistance of the liquids and operates a device for controlling the fat content of the cream. R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

310. Terramycin supplement for dairy calves. A. M. MACKAY, W. H. RIDDELL, and R. FITZSIMMONS. Vt. Agr. Expt. Sta. Progress Rept. Feb., 1953.

Eighteen (10 experimental, 8 control) heifer calves in the College herd were fed terramycin hydrochloride at the rate of 30 mg. per 100 lb. live weight for a 12-wk. period. The calves received a liberal ration of milk, calf starter, and good quality hay. Comparison of the weights of the two groups showed both to exceed the Ragsdale standard, the supplemented calves by an average of 40.0 lb., or 20%, and the controls by 25.9 lb., or 13.6%. The calves receiving terramycin showed a smoother coated appearance and had a higher level of calf starter consumption.

Feeding the supplement to older calves, starting at 6 to 9 wk. of age, produced less effect on growth. There was an initial increase over the controls in the first 2 wk., but at the end of 8 wk. of feeding little difference was noted in rate of gain compared with the controls. W. H. Riddell

311. Effects of prolonged percutaneous administration of methyl testosterone and estradiol on growing male rats. A. E. LIGHT and J. A. TORNABEN. J. Nutrition, **49**, 1: 51-61. Jan., 1953.

One group of growing male rats were inucted daily with an ointment containing 30 γ of estradiol and another group were inucted with an ointment containing 400γ of methyl testosterone. The estradiol-inucted rats showed severe retardation of growth and development and increased water and food intake but with low efficiency in adding muscle and fat tissues. Skeletal growth increased, but the rate of healing of wounds was slower and urinary protein was reduced. Food and water consumption increased when these animals were exposed to a temperature of 22.5° C. and then decreased when they were returned to an environment of 28.5 °C. The inuction of methyl testosterone was associated with enlargement of the kidneys, possible increased disposition of protein tissue, and a shortened time required for wound healing. Figures are presented showing the preliminary results of histopathological studies of the spleen, adrenal cortex, and testes from animals given the androgen and estrogen treatments. O. T. Stalleup

312. The metabolic fecal nitrogen excretion of the young calf and the true digestibility of casein. G. P. LOFGREEN and M. KLEIBER. J. Nutrition, **49**, 1: 183-190. Jan., 1953.

Data are presented concerning the fecal nitrogen excretion of young dairy calves fed casein labeled with radioactive P^{32}. The true digestibility of the casein nitrogen averaged 93.5%, and the average metabolic nitrogen excretion was was 0.27 g. per 100 g. dry matter intake. O. T. Stalleup

313. A quantitative study of rumen synthesis in the bovine on natural and purified rations. II. Amino acid content of mixed rumen proteins. C. W. DUNCAN, I. P. AGRAWALA, C. F. HUFFMAN, and R. W. LUECKE. J. Nutrition, **49**, 1: 41-49. Jan., 1953.

Rumen contents from calves fed a purified ration in which urea supplied the only source of dietary nitrogen and from one calf on a

natural ration were assayed microbiologically for amino acid content. The amino acid pattern of the mixed protein of the ingesta of the calves on the purified diet was similar to that found in the calf fed the natural ration, thus indicating that the microorganisms can utilize urea nitrogen to synthesize amino acids. O. T. Stallcup

314. A quantitative study of rumen synthesis in the bovine on natural and purified rations. I. Protein, dry matter and non-protein nitrogen. I. P. Agrawala, C. W. Duncan, and C. F. Huffman. J. Nutrition, **49**, 1: 29-39. Jan., 1953.

Balance trials were conducted with 3 rumen-fistulated calves on both a natural ration and a purified ration, in which urea supplied the only essential source of nitrogen, in order to determine the rate of disappearance of dry matter and NPN from the rumen and the biosynthesis of protein. Rumen dry matter and NPN increased percentagewise within 6 hr. after feeding. Crude protein increased in only 4 out of 7 trials. The rate of removal of dry matter from the rumen was 60% and of NPN 90% within 6 hr. after feeding. An appreciable synthesis of protein assumed to be due to the activity of microorganisms was observed within 6 hr. after feeding. O. T. Stallcup

315. Dermatosis in weanling rats fed lactose diets. II. Histological studies. H. Nino-Herrera, N. Schreiber, R. A. Collins, and C. A. Elvehjem. J. Nutrition, **49**, 1: 99-105. Jan., 1953.

Histological data are presented showing changes in the skin from the dorsal side of the hind paws of weanling rats fed cow's milk to which Fe, Cu, and Mn were added. Similar changes occurred at both high and low relative humidity. The principal change in the skin was a hyperkeratinization of the hair follicles. Scaliness was observed at low, but not at high, relative humidity. O. T. Stallcup

316. Response of cobalt-deficient sheep to intravenously administered Vitamin B_{12}. W. G. Hoekstra, A. L. Pope, and P. H. Phillips. J. Nutrition, **48**: 431-441. Dec., 1952.

Three cobalt-deficient sheep were given intravenous injections of 20 γ of Vitamin B_{12} twice daily for 21 days. This treatment resulted in increases in feed consumption, vigor, and body weight gains. Blood hemoglobin levels rose to normal in 40 to 60 days. This response to treatment lasted for about 100 days, after which time a second cobalt deficiency developed. Two of the sheep were given a second series of injections and again exhibited similar responses. The authors suggest that Vitamin B_{12} is the most important, if not the only, limiting factor in cobalt-deficient sheep. O. T. Stallcup

317. Synthesis of certain B-vitamins in the cobalt deficient sheep with special reference to vitamin B_{12}. W. G. Hoekstra, A. L. Pope, and P. H. Phillips. J. Nutrition, **48**: 421-430. Dec., 1952.

The blood of sheep early in cobalt deficiency contained one-fifth the Vitamin B_{12} activity of the blood from cobalt-fed sheep. Liver storage of Vitamin B_{12} was about 30 times as great in cobalt-fed sheep as compared to those fed cobalt-deficient rations. Rumen synthesis of Vitamin B_{12} was 15 times as great when sheep were given cobalt as compared to those on a deficient diet. Rumen synthesis of nicotinic acid and riboflavin was essentially the same per gram of dry rumen material in deficient and cobalt-fed animals. O. T. Stallcup

SANITATION AND CLEANSING

K. G. Weckel, Section Editor

318. Relative toxicity of furethrin, its natural acid isomer, and pyrethrins to house flies. W. A. Gersdorff and N. Mitlin, Bur. of Entomol. and Plant Quarantine, U.S.D.A. J. Econ. Entomol., **45**, 5: 849-850. Oct., 1952.

Laboratory turntable tests were made with refined kerosene solutions of 3 insecticides to determine their effectiveness in knockdown and kill of the house fly when used at concentrations of from 0.5 to 8 mg. per ml. Furethrin, an analog of allethrin, was only slightly more toxic than pyrethrins. The d-trans chrysanthemum monocarboxylic acid isomer of furethrin was about twice as toxic as furethrin. All materials had high knockdown value. E. H. Fisher

319. Continuous vaporization of insecticides with special reference to DDT. P. J. Spear and H. L. Sweetman, Univ. of Mass., Amherst. J. Econ. Entomol., **45**, 5: 869-873. Oct., 1952.

Constant, uniform vaporization of an insecticide such as DDT may control several species of insects in complete, or nearly complete enclosures. Under favorable circumstances, 1 g. DDT per 25,000 cu. ft. per day is sufficient —this amount per 15,000 cu. ft. is better.

Evaporation rates are affected directly by surface area of insecticide exposed and by temperature. Temperature control of vaporizing units should be adjustable for adaptation under variable conditions, including those of air rate change and size of enclosure. DDT, with a low vapor pressure, condenses into aerosol-size liquid droplets immediately after being vaporized. Air currents disperse these droplets up to 4 to 5 days, after which time they crystallize on some solid surface.

Vaporization of insecticide has been useful in more or less enclosed areas against many flying insects and some crawling insects. E. H. Fisher

320. Synergists for DDT against insecticide-resistant house flies. R. B. March, R. L. Metcalf, and L. L. Lewallen, Univ. of Calif.,

Riverside. J. Econ. Entomol., **45**, 5: 851-860. Oct., 1952.

Sixteen of 96 compounds structurally related to DDT were proven synergistic to DDT against DDT-resistant house flies. No such combination was as effective against resistant flies as DDT alone against susceptible flies. Similar, but lesser, synergism was found for methoxychlor. There was no synergism of DDT against DDT-susceptible flies. Synergized DDT or methoxychlor preparations were about as effective in knockdown against resistant flies as was DDT or methoxychlor alone against susceptible flies, but resistant flies required 2 to 6 times as long exposure to deposits of the combinations as susceptible flies required with DDT or methoxychlor alone. A topical application of microdrops to individual flies was the procedure to evaluate synergism.

Insecticide-resistant flies in areas of high breeding, such as hog farms, were controlled effectively for 6 to 8 wk. by applications of 20 lb. DDT plus 4 to 8 lb. synergist per 100 gal. of spray. Effectiveness was somewhat less in dairies. A high-pressure orchard-type sprayer was used to apply insecticide to the run-off point.

As great resistance to synergized DDT as to DDT alone developed quickly in the laboratory.

E. H. Fisher

321. Study substantiates benefits of cleaning pipelines in place. R. B. Parker, P. R. Elliker, G. T. Nelson, G. A. Richardson, and G. H. Wilster, Ore. Agr. Expt. Sta., Corvallis. Food Eng., **25**, 1: 82-86, 176, 177, 178. Jan., 1953.

A 12-mo. investigation of recirculating cleaning procedures was made in a commercial plant with 300 ft. of pasteurized, and 750 ft. of raw, milk piping. The lines included some glass sections. Dismantling was done only for inspection and replacement of gaskets. The water was soft, approximately 6 gr./gal., or 105 p.p.m. as $CaCO_3$. General cleaning procedures included a circulating pre-rinse, a circulating hot, alkaline detergent wash, a fresh water rinse, and a sanitizing treatment with 200 p.p.m. hypochlorite. Valve plugs and seats were specially treated. A special tank unit with automatic controls and recording devices was developed to circulate the solutions. Bacteriological examinations were made of the lines weekly with a swab method. Of 14 different detergents studied, best results were obtained using a highly alkaline cleaner with relatively high wetting agent content at 170° F. Glass sections cleaned slightly easier than stainless steel. Lines should be sloped for self-drainage, and some back pressure should be applied on the circulating solutions. Neoprene and Teflon gaskets were satisfactory. All cleaning procedures required approximately ¼ the time of take-down methods. Bacteriological condition of the lines was always excellent and superior to that under conventional procedures.

T. J. Claydon

322. Fountain brush for sanitary pipe washing equipment. P. K. Girton. U. S. Patent 2,626,413. 1 claim. Jan. 27, 1953. Official Gaz. U. S. Pat. Office, **666**, 4: 931. 1953.

A brush with tufted bristles set in a closed tubular head for cleaning sanitary pipe. The brush head is threaded and attached to a pipe or hose. Water or cleaning solution is delivered through holes in the head. R. Whitaker

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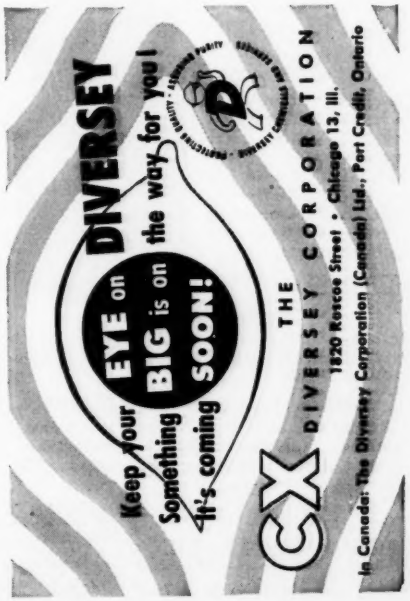
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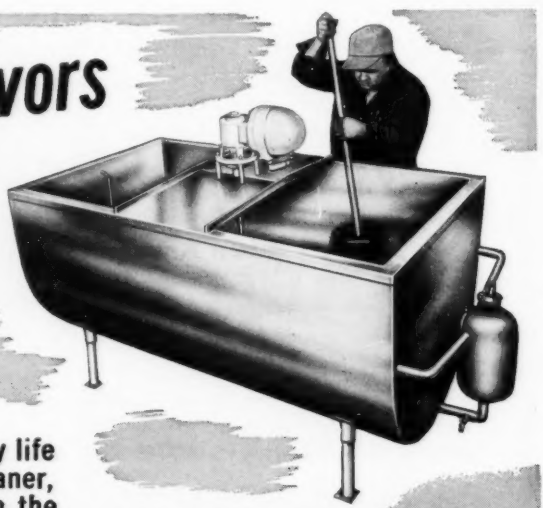

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